A PILOT STUDY OF SERUM GRANULYSIN IN DRUG INDUCED EXANTHEMS AND SEVERE CUTANEOUS ADVERSE REACTIONS (SCARs)

A dissertation submitted in partial fulfillment of the rules and regulations for M.D (Dermatology, Venereology and Leprosy) examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai, to be held in May, 2018.

DECLARATION

I hereby declare that the dissertation entitled **"A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)**" conducted at Christian Medical College, Vellore is my original research work done in partial fulfillment of the rules and regulations for the award of the M.D. degree in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R. Medical University.

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CERTIFICATE

This is to certify that the dissertation entitled **"A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)**" conducted at Christian Medical College, Vellore is a bonafide research work done by **Dr. Jacquline Jose.** This study was undertaken at Christian Medical College, Vellore from **November, 2015 to May, 2017** under my direct guidance and supervision, in partial fulfillment of the requirement for the award of the M.D. degree in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R. Medical University.

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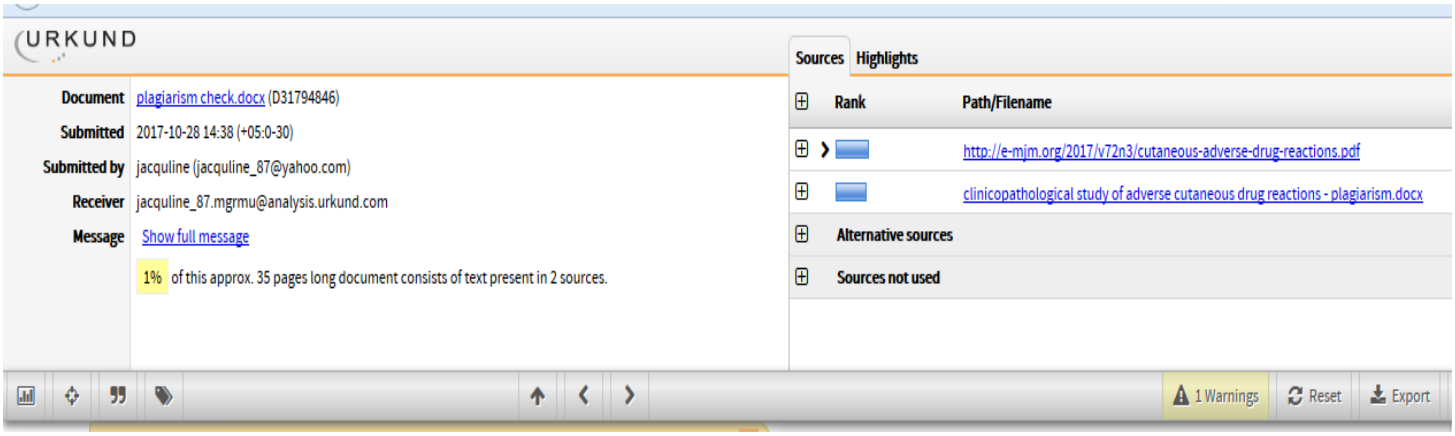

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ABBREVIATIONS

ADR	Adverse drug reaction
AGEP	Acute generalised exanthematous pustulosis
CADR	Cutaneous adverse drug reaction
CMV	Cytomegalovirus
CTL	Cytotoxic T cells
DIHS	Drug induced hypersensitivity syndrome
DRESS	Drug reaction with eosinophilia and systemic symptoms
EBV	Epstein Barr virus
EMM	Erythema multiforme major
FDE	Fixed drug eruption
GBFDE	Generalised bullous fixed drug eruption
GVHD	Graft versus host disease
HIV	Human immunodeficiency virus
HHV	Human herpes virus
HLA	Human leukocyte antigen
IFN	Interferon

IL	Interleukin
IVIG	Intravenous immunoglobulin
LTT	Lymphocyte transformation test
MCD	Mast cell degranulation test
MIF	Macrophage migration inhibition factor test
MHC	Major histocompatibility complex
MPE	Maculopapular exanthema
NKTL	Natural killer T lymphocytes
NSAID	Non-steroidal anti-inflammatory drugs
ODSR	Ordinary drug induced skin reaction
SCAR	Severe cutaneous adverse reaction
SJS	Stevens – Johnson syndrome
TEN	Toxic epidermal necrolysis
TNF	Tumour necrosis factor

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INTRODUCTION

Severe cutaneous adverse events are one of the dermatological emergencies. The clinical presentation of cutaneous adverse drug reactions (CADRs) could range from self-limiting maculopapular exanthema (MPE) to life threatening toxic epidermal necrolysis (TEN). Fortunately 90% of these are benign maculopapular eruptions which subside within a few days of discontinuation of the drug (1), without any significant long term complications. However, approximately 1: 1000 hospitalized patients with cutaneous adverse drug reactions also manifest systemic features such as fever, lymphadenopathy and visceral involvement. This accounts for a significant burden on health care costs.

There is a lack of conclusive diagnostic tests available for confirming the cutaneous adverse drug reactions (CADRs). Diagnosis is often clinical. There are many criteria which have been proposed for the diagnosis of severe cutaneous adverse reactions (SCARs). The dilemma arises when a SCAR presents with a rash reminiscent of a maculopapular exanthema (MPE) in the early phase of the illness. Systemic involvement may not be apparent at this stage. In the absence of adequate follow up, misdiagnosing the rash as an ordinary drug induced skin reaction (ODSR) can have grave consequences.

In this setting, identification of biochemical markers that can promptly assess the potential risk of a rash progressing to a SCAR, looks promising. Granulysin is a cytolytic granule protein produced by cytolytic T cells and natural killer cells which plays a crucial role in immunity (2). It has recently garnered attention due to its role in the pathogenesis of Stevens – Johnson syndrome and Toxic

epidermal necrolysis. However, there are few studies that have studied its role in other CADRs. Despite extensive literature search, we did not find any Indian studies that have studied the role of granulysin in cutaneous adverse drug reactions.

In this proposed study, we aim to measure the serum granulysin in patients with different CADRs including SCARs and non-severe reactions such as MPE, and correlate it with the clinical severity of the disease. We hope to identify the utility of serum granulysin as a rapid diagnostic test for CADRs and as a novel therapeutic target for the management of severe adverse reactions.

AIMS AND OBJECTIVES

1. To measure the serum levels of granulysin and study its relationship with disease severity in patients with drug induced exanthemas and severe cutaneous adverse reactions.
2. To study the clinical and cutaneous profile of patients with drug induced exanthemas and severe cutaneous adverse reactions.

REVIEW OF LITERATURE

I.INTRODUCTION

Edwards and Aronson defined adverse drug reaction as “an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dose regimen, or withdrawal of the product”(3).

A hospital- based surveillance by the ‘WHO International Programme for Adverse Reaction Monitoring’ identified skin as the most frequently affected organ in adverse drug reactions (4,5). They account for 3-6% of all hospital admissions and 5-9% of hospital admission costs (6). Therefore they are a big burden on the public healthcare system, especially in low income countries. Studies on large cohorts of hospitalized patients have estimated the incidence of cutaneous adverse reactions to be approximately 2% - 3% (7–9). The frequency of cutaneous adverse reaction to specific drugs vary from 1% to 10% (1,5). While most drug related adverse events are maculopapular exanthema (MPE) (7,10,11) that closely mimic viral illnesses, some of the more severe adverse drug reactions can be fatal.

II.CLASSIFICATION OF ADVERSE DRUG REACTIONS BASED ON PATHOGENESIS

Adverse drug reactions can be broadly classified as :

1. Non- immunologic response
2. Immunologic response

The non-immunologically mediated reactions constitute 80-90% of the adverse drug reactions (ADRs). They are usually dose dependent and predictable. For instance, diarrhea after ingestion of antibiotics and mucositis with chemotherapeutic agents or hepatotoxicity with methotrexate (12,13). They are related to the pharmacological activity of the drug.

In contrast, the immunologically mediated reactions are non dose-dependent and unpredictable. Approximately 90% of immunologically mediated reactions are maculopapular exanthema (14), but around 1 in 1000 hospitalized patients with adverse drug reactions are estimated to develop severe reactions (1). They result from a delayed type IV hypersensitivity response to the drug **(fig 1)**. These can develop virtually with any drug, but few groups of drugs are more notorious for causing immunologically mediated drug reactions.

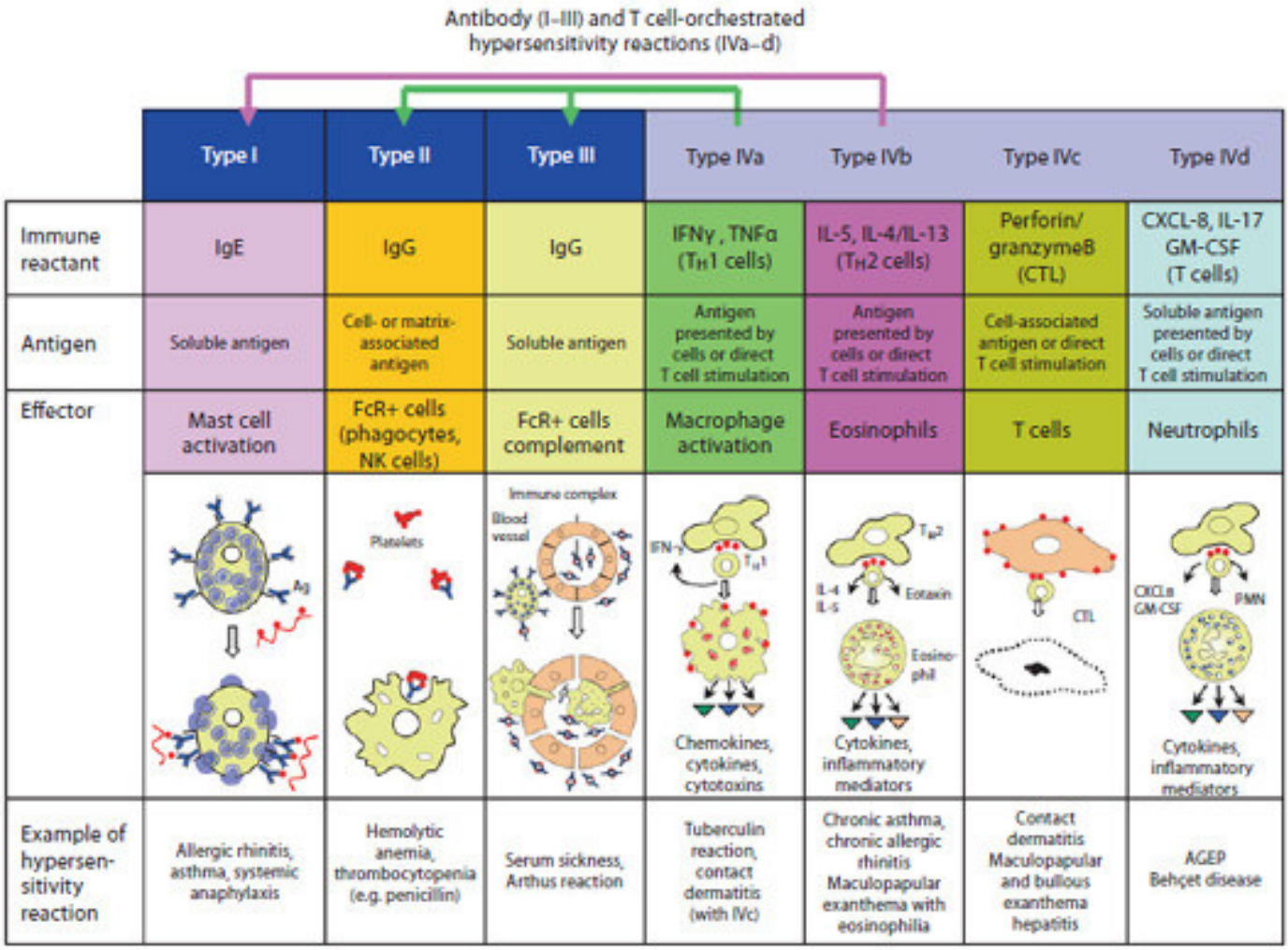


Figure 1- Revised Gell and Coombs classification of hypersensitivity reactions

(Adapted from Pichler WJ. Drug hypersensitivity : classification and relationship to T-cell activation)

It has been noted that maculopapular or morbilliform eruptions usually occur 9 ± 5 days after initiation of the drug, whereas most severe reactions have a longer latent period - 14 ± 7 days for Stevens - Johnson syndrome/ Toxic epidermal necrolysis (SJS/TEN) and 28 ± 14 days for drug reaction with eosinophilia and systemic symptoms (DRESS) (15). However, a rechallenge with the same drug

induces recurrences sooner. This suggests that the drug reaction is mediated by sensitization and a specific immunological memory rather than a direct toxic effect (15).

Acute generalized exanthematous pustulosis (AGEP) and fixed drug eruptions (FDE) manifest within 1-3 days. This is hypothesized to be due to recall phenomenon, secondary to an overt or latent exposure to the offending drug (15).

III.SEVERE CUTANEOUS ADVERSE REACTIONS

The World Health Organization defines a severe cutaneous adverse reaction as one 'that requires hospitalization or prolongation of the current hospital admission, causes significant or persistent disability and puts life in danger or causes death'(3). A meta-analysis of prospective studies conducted in the United States of America showed that 6.7 % of drug reactions were SCARs (16) with a fatality rate of 0.32%. Severe cutaneous adverse reactions are a group of potentially life threatening drug-induced systemic illnesses that occur as a result of complex patho-mechanisms and genetic predisposition.

Severe cutaneous adverse reactions include the following entities :

1. Stevens-Johnson syndrome (SJS) – Toxic epidermal necrolysis (TEN) spectrum
2. Drug reaction with eosinophilia and systemic symptoms (DRESS)
3. Acute generalised exanthematous pustulosis (AGEP)

While most maculopapular exanthemas and urticaria are secondary to antibiotics, severe cutaneous adverse reactions are usually secondary to drugs such as anticonvulsants and allopurinol (17). They can

also result in long term sequelae, thus affecting the quality of life of the surviving patients. It is of utmost importance to identify the offending drug and withhold it as early as possible, as this affects the outcome of the patient.

1.STEVENS - JOHNSON SYNDROME / TOXIC EPIDERMAL NECROLYSIS

In 1922, Stevens and Johnson used the term ‘Stevens – Johnson syndrome’ to describe the clinical syndrome of fever with a disseminated erythematous macular rash with a necrotic center, severe stomatitis and conjunctival involvement in two children. Later, Lyell coined the term ‘Toxic Epidermal Necrolysis’ to describe chafed-looking skin lesions in four patients, which he believed to be toxin-mediated (18). ‘Necrolysis’ referred to the histopathological finding of epidermal necrosis in this syndrome. He subsequently identified a higher incidence of the above skin changes in patients who were on sulfonamides, antiepileptic agents and pyrozolones.

It is now well known that SJS and TEN belong to a spectrum of disorders which only differ by the area of skin detachment. Stevens - Johnson syndrome presents with epidermal detachment involving less than 10% of the body surface area, whereas TEN refers to the most severe form with detachment of more than 30%. The intermediate variant is called SJS-TEN overlap. Erythema multiforme major and SJS were historically considered to belong to the same spectrum (19), but are now regarded as being clinically and aetiologically distinct (20).

1.1 Epidemiology of SJS/TEN : The annual incidence of SJS and TEN is estimated to be 1.2-6 cases per million population and 0.4-2 cases per million population respectively (21). However, actual numbers vary based on a number of factors such as age, gender, ethnicity, and prescription trends. Medications are responsible for around 80% of TEN and 50% of SJS cases (13). The drugs commonly implicated in SJS/TEN are shown in **table 1**. The estimated mortality rates are approximately 5% for SJS and between 30 to 50% for TEN (22).

Table 1 - Drugs commonly implicated in SJS/TEN

Antibiotics	Penicillins Cephalosporins Fluroquinolones Sulfa drugs Antimalarials
NSAIDs	Paracetamol Ibuprofen Nimesulide Mefenemic acid
Antiepileptics	Phenytoin carbamazepine
Others	ART Alternative medicines

Adapted from Sethuraman et al (23)

1.2 Pathogenesis of SJS/TEN :

Many HLA haplotypes such as HLA-B*1502, HLA-B*5801and HLA-B*5701 have been demonstrated to have susceptibility to develop toxic epidermal necrolysis after the administration of carbamazepine, allopurinol and abacavir respectively among Han Chinese population (24,25). Literature indicates that HLA-DQB1*0601 is associated with ocular complications in SJS in Caucasian patients (26). Yet another study indicates that HLA B12 is expressed in patients with TEN (27). Thus, HLA typing in patients among the at-risk ethnic groups plays a major role in prevention of CADRs.

The widely accepted theory for pathogenesis of SJS/TEN states that in response to the drug or its metabolites, there is a cutaneous recruitment of antigen-primed CD8+ cytotoxic T lymphocytes (CTLs) and CD56+ natural killer cells (NK cells), directed against the keratinocytes in a major histocompatibility class (MHC) I - restricted manner (28,29). This keratinocyte apoptosis is mediated by granulysin, perforin and granzyme B which are present in the granules within CTLs and NK cells (30). It is noteworthy that these molecules are elevated in the blister fluid in SJS/TEN. Granulysin is exocytosed along with perforins which facilitates its entry into the keratinocyte. Thereafter it damages the cell membrane and disrupts the mitochondrial transmembrane potential and causes disseminated keratinocyte death **(fig 3)** (30).

The alternate theory proposes that Fas - Fas Ligand signaling pathway produces caspase 8 activation, which induces cell apoptosis (31,32). This mechanism is induced by cytokines and other soluble factors such as tumour necrosis factor α (TNF-α), Interferon γ (IFN-γ) , Interleukin 8 (IL-8) and nitric oxide (33).

Rechallenge with an offending drug typically shortens the incubation period and produces more severe clinical manifestations (15). Thus, educating the patient on the need for avoidance of re-exposure to the drug is crucial.

1.3 Clinical features of SJS/TEN :

After the maiden exposure to the drug, the skin and systemic symptoms commence within 7-21 days (34). There is a prodrome of fever, myalgia, arthralgia, anorexia, rhinorrhea and a pricking sensation of mucocutaneous surfaces. In the initial stage, the skin manifestation are composed of pruritic maculopapular, urticarial, petechial or purpuric rashes. Lesions are bilaterally symmetrical and generally start on the trunk, followed by involvement of the head and neck and proximal aspects of extremities. Distal extremities, palms and soles are rarely involved (34). These may evolve into target-like lesions, which may rapidly develop into vesicles and bullae. This results from epidermal keratinocyte necrosis and subsequent sub-epidermal detachment. Nikolsky and Asboe-Hansen signs are positive. The denuded dermis appears shiny and erythematous with pinpoint bleeding spots. On an average, this evolution of symptoms occurs in 6-9 days (35).

Mucosal involvement is seen in 90% patients with SJS/TEN and they tend to appear early in the course of the disease. This is predictive of a higher risk of progression of SJS to TEN (1). Ocular mucosal involvement, ranging from hyperemia to corneal rupture, is seen in 85% and genital involvement is seen in 40-60% patients. All three mucosae may be involved in up to 50% patients (34).

Often, SJS/TEN are complicated by end organ damage affecting the renal, pulmonary, gastrointestinal and cardiovascular systems. Renal damage occurs as a consequence of hypovolemia, decreased cardiac output and cytokine induced nephrotoxicity. It may present as dyselectrolemia, pre-renal hyperazotemia, renal tubular necrosis and acute renal failure (36). Bronchiolitis obliterans and diffuse interstitial pneumonitis may develop and therefore it is paramount to closely monitor for the same even if initial chest radiographs are normal.

1.4 Scoring system :

Severity of TEN can be assessed by SCORTEN scale (37), which is an aggregate score based on seven independent parameters that can adversely affect the outcome in the patient **(table 2).** In addition, it is a useful tool for prognosis.

Table 2 - SCORTEN scale for assessment of severity of TEN

S.no	Variable	Values	Score
1	Age	≥ 40 years	1
2	Heart rate	≥ 120 bpm	1
3	Malignancy		1
4	Initial epidermolysis	≥ 10% BSA	1
5	Serum urea	≥ 10 mmol/L	1
6	Serum bicarbonate	< 20 mmol/L	1
7	Serum glucose	≥ 14 mmol/L	1

Adapted from Bastuji – Garin et al (37)

SCORTEN score is calculated within 24 hours of admission and repeated on the third day. The risk of mortality increases with SCORTEN score, and the predictive value is best when calculated on the third day of admission (38). Mortality prediction based on the SCORTEN score is represented in **table 3.**

Table 3 – Mortality prediction based on SCORTEN score

SCORTEN score	Percentage of mortality
1	3.2 %
2	12.1 %
3	35.8 %
4	58.5 %
≥ 5	90%

1.5 Management of SJS/TEN:

Management of SJS/TEN is largely supportive. This includes discontinuation of the culprit drug, admission in an intensive care facility for fluid and electrolyte management, thermoregulation, adequate nutrition, analgesia and prevention of secondary infections. The main cause of mortality is secondary infection leading to sepsis. Therefore it is essential to provide reverse barrier nursing and antimicrobial therapy as deemed fit by the clinician. Re-epithelialization occurs by the migration of keratinocytes from the follicular reserves and recovery occurs within 3 weeks (34).

The cutaneous sequelae of SJS/ TEN include dyspigmentation of skin, alopecia, hypohidrosis or anhidrosis, scarring and nail changes, to name a few. More significantly, these patients may also develop mucosal changes, most common of which are ocular changes like chronic conjunctivitis, pseudomembrane formation and cataracts. Ectropion, corneal scarring and subsequent loss of vision are dreaded complications which need to be prevented by proactive therapy during the acute phase of the disease. Long term complications of the respiratory tract may lead to chronic bronchitis, bronchiectasis and bronchiolitis obliterans. Similarly, involvement of the gastrointestinal tract and genitourinary tract may lead to mucosal strictures. Hypopharyngeal stenosis, dental hypoplasia, glomerulonephritis and idiopathic thrombocytopenic purpura have also been described. Finkelstein et al reported relapse of SJS/TEN in 7.2% patients with a mean duration of 315 days between the episodes (39). The cause of the relapse was proposed to be cross-reactivity with drugs with a similar chemical structure, and genetic susceptibility.

There are contradicting schools of thought regarding the use of systemic agents in the acute phase of the disease. Till the early 1990s, systemic steroids were the accepted standard of treatment for SJS/TEN. However, since then it has been shown to increase the risk of complications such as sepsis and gastrointestinal bleeding (40). High dose pulse steroid therapy has been proposed as an alternative to reduce these complications. Steroids suppress the release of cytokines and inhibits T cell and Fas ligand mediated cell death.

Many other systemic agents have been tried in the treatment of SJS/TEN. Cyclosporine inhibits CTLs by suppressing IL-2 production and thereby decreases the levels of granulysin (41). Intravenous

immunoglobulin was successfully used for a series of 10 patients with TEN in 1998 (42). It is thought to inhibit the Fas ligand pathway mediated keratinocyte apoptosis. Other regimes that have been used for the management of SJS/TEN are corticosteroids or infliximab in combination with Intravenous immunoglobulin (IVIG). N-acetylcysteine is a cysteine derivative with antioxidant properties and a capacity to inhibit TNF-α and IL1β. In addition, anti-TNF α blockers including biologics, pentoxiphylline and plasmapheresis have been used for treatment of SJS/TEN.

2.DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS (DRESS)

The term 'Drug reaction with eosinophilia and systemic symptoms' to describe the syndrome of a cutaneous adverse reaction with lymphadenopathy, haematological derangements and systemic features was introduced by Bocquet et al in 1996 (43). Prior to this, several other terminologies such as 'hypersensitivity syndrome', 'drug induced delayed multi-organ hypersensitivity syndrome', 'drug induced hypersensitivity syndrome', 'anticonvulsant hypersensitivity syndrome' and 'febrile mucocutaneous syndrome' were variably used to describe this constellation of clinical features. There are a limited number of drugs suspected to cause DRESS syndrome. The drugs most commonly implicated are anticonvulsants, dapsone, allopurinol, minocycline, sulfasalazine and abacavir (44,45).

Another characteristic feature of DRESS is the delay in onset of symptoms. The time duration between the initiation of the drug and the first cutaneous manifestation may range from 3-8 weeks (45). This presents a diagnostic challenge unless the index of suspicion is high. Other severe cutaneous adverse reactions have a shorter latent period and symptoms resolve more rapidly (46). Yet another factor

which compounds this difficulty in diagnosis is the clinical similarity of DRESS and infectious mononucleosis like syndrome (47). Infection with human immunodeficiency virus, Hepatitis A virus, Hepatitis B virus and Influenza virus may also mimic DRESS syndrome (46).

2.1 Pathogenesis of DRESS syndrome :

The exact mechanism of DRESS is not fully understood, but three main components are thought to be involved.

1. Abnormalities in metabolic pathways resulting in the accumulation of reactive metabolites (such as a deficiency or abnormality in epoxide hydroxylase, an enzyme that detoxifies the metabolites of aromatic amine anticonvulsants) ;
2. Associated sequential reactivation of herpesvirus family members ;
3. Ethnic predisposition in people with certain HLA alleles (immune response) (15,30,31,36).

Saito et al proposed that granulysin is secreted against the virus infected cells in DRESS. This hypothesis explains the prolonged elevation of serum granulysin in DRESS, which coincides with the reactivation of herpes viruses (47).

2.2 Clinical features of DRESS syndrome :

Cutaneous changes are composed of urticated or infiltrated papules, and less commonly, vesiculo-bullous lesions, pustules or purpura. The skin lesions may further progress to erythroderma (48). The cutaneous eruption may also mimic AGEP or EMM. However, a particular morphology of rash is not required for the diagnosis as it is based on the characteristic clinical course, organ involvement and

human herpes virus (HHV-6) reactivation. DRESS syndrome with skin features similar to SJS/TEN have been reported, in which the diagnostic criteria of DRESS syndrome is fulfilled but patients in addition exhibit epidermal detachment and mucosal erosions characteristic of SJS/TEN. Patients with DRESS syndrome are typically febrile with lymphadenopathy, leukocytosis, peripheral eosinophilia and deranged liver function tests. Facial or periorbital oedema may be present (49), in addition to interstitial inflammation of the liver or kidneys (44,45).

There is a chronic clinical course in DRESS as compared to the other SCARs due to the high frequency of relapse. There may be reactivation of HHV-6 two to three weeks after the onset of the symptoms, which presents as fever and hepatitis. There have been reports of reactivation of other herpes viruses including HHV-7, cytomegalovirus (CMV) and Epstein Barr virus (EBV). Among these, CMV reactivation is associated with recurrent transient fever, cutaneous eruption and systemic complications such as myocarditis, pneumonitis or gastrointestinal bleeding (48). Epstein Barr virus and HHV-7 reactivation are thought to have no clinical implications. It has been postulated that there is a transient drug-induced hypogammaglobulinemia during the acute phase of DRESS syndrome which enables viral reactivation (50). The estimated mortality rate of DRESS is approximately 10%. The most common cause of death is acute liver failure (51,52).

The chronology of events in DRESS is depicted in the figure below **(fig 2).**

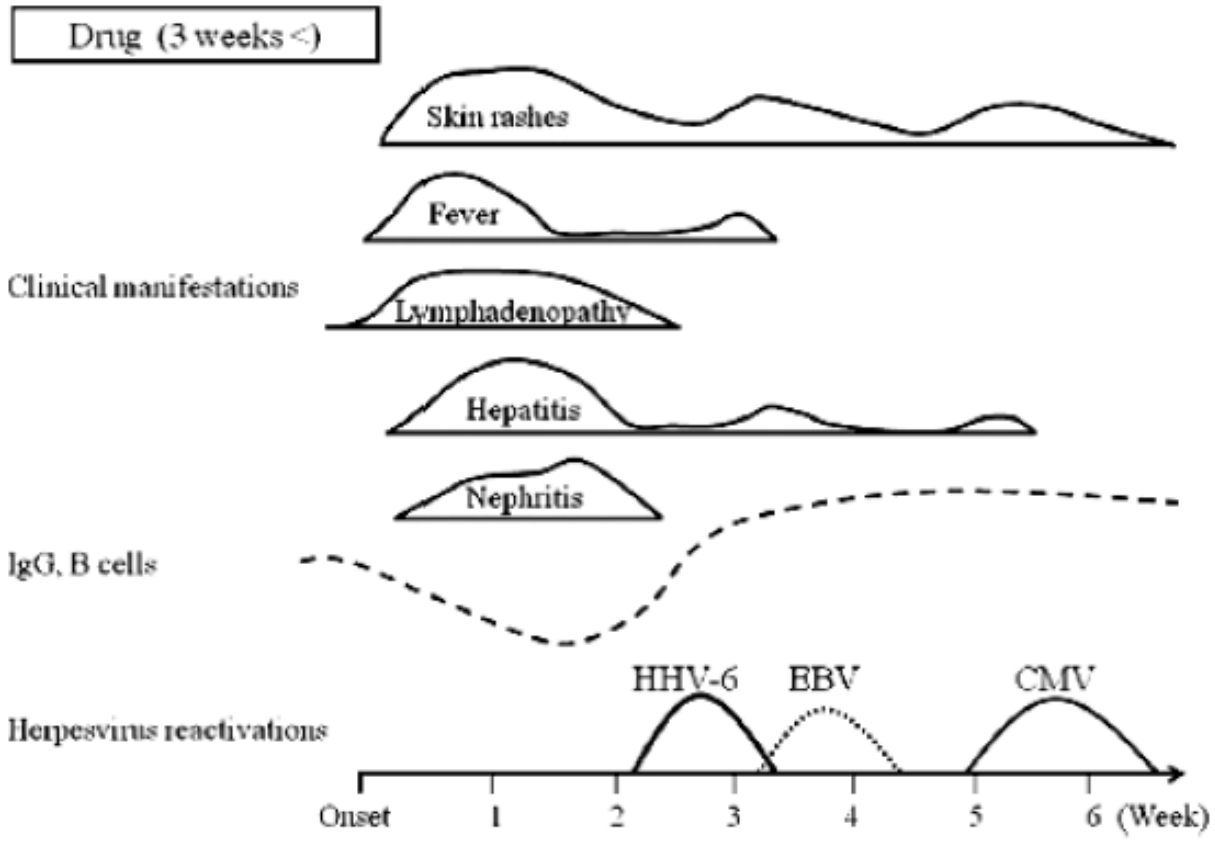


Figure 2 – Chronology of events in DRESS

2.3 HLA associations in DRESS syndrome :

The extent of skin involvement varies from patient to patient and this is probably a reflection of genetic susceptibilities and environmental factors. For instance, it has been demonstrated that there is a strong association between HLA-B*5801 and allopurinol induced DRESS and SJS in the Han Chinese population(24).

2.4 Criteria for DRESS syndrome :

The RegiSCAR criteria was formulated to aid in the diagnosis of DRESS syndrome in view of its multitude of presentations (53) – **Annexure 6.**

Another diagnostic criteria for Drug induced hypersensitivity syndrome (DIHS) in use is the Japanese consensus group criteria, which includes the following :

1. Maculopapular rash developing after 3 weeks of initiation of the suspected drug
2. Symptoms persisting beyond 2 weeks of stopping the drug
3. Fever >38 degree Celsius
4. Liver abnormalities (ALT>100U/L) or other organ involvement
5. Leukocyte abnormalities
    a) leukocytosis > 11000/ml
    b) atypical lymphocytosis >5%
    c) eosinophilia > 1500/ml
6. Lymphadenopathy
7. Human herpes virus 6 reactivation

The diagnosis is confirmed by the presence of all 7 of the above criteria (typical DIHS) or the first 5 of the 7 criteria (atypical DIHS). DIHS is believed to be a subset of DRESS which is more severe and associated with HHV-6 reactivation (48).

It is a daunting task to determine the drug responsible for the adverse reaction, especially in patients with a history of polypharmacy. In DRESS syndrome, patch testing and lymphocyte transformation testing (LTT) have been used for this purpose with reasonable success (46).

2.5 Differential diagnosis :

Differentials for DRESS syndrome should include viral exanthema, vasculitides and other haematological and lymphocytic conditions. Among vasculitides, polyarteritis nodosa (PAN), granulomatous polyangiitis (GPA), eosinophilic granulomatous polyangiitis (EGPA), systemic lupus erythematosus (SLE), Kawasaki disease and Still's disease are known to cause skin manifestations similar to DRESS syndrome. Angioimmunoblastic lymphadenopathy (a subtype of peripheral T cell lymphoma), lymphoma, pseudolymphoma and idiopathic hypereosinophilic syndrome are the other diseases to be considered when evaluating a patient with suspected DRESS syndrome (46).

2.6 Management of DRESS syndrome :

Once the clinical diagnosis of drug reaction with eosinophilia and systemic symptoms is suspected, the offending drug has to be stopped immediately to prevent further compromise of the visceral organs. Patients with DRESS syndrome complicated by exfoliative dermatitis should be admitted in an intensive care or burns unit for further supportive care and there should be a low threshold for initiation of systemic steroids (46). It is recommended that systemic steroids be gradually tapered and stopped, unlike in SJS/TEN with close monitoring of haematological, renal and hepatic parameters.

3.ACUTE GENERALISED EXANTHEMATOUS PUSTULOSIS

In 1968, Baker and Ryan described a group of patients with a pustular dermatosis who had an acute course and resolution, absence of history of psoriasis and in whom the episodes did not recur (54). They used the term ‘exanthematic pustular psoriasis’ to describe this constellation of features and suspected the condition to be triggered by drugs or infection. Meanwhile, the terms ‘toxic pustuloderma’ (55) and ‘pustular drug rash’ (56) were used to describe patients with a similar symptomatology. The current term was introduced by Beylot et al in 1980.

3.1Clinical features of AGEP :

Acute generalised exanthematous pustulosis is a cutaneous adverse reaction characterized by sheets of sterile pinpoint non-follicular pustules on an erythematous oedematous base, with a predilection for the face and flexural areas (57–59). The diagnosis is largely uncomplicated due to the distinct morphology

of the skin lesions. The latent period is short and ranges between two to five days. Confluent pustular lesions may show epidermal detachment mimicking TEN, however, this is rare (57). Mucosal involvement is uncommon though cheilitis may be seen in around 20% of the patients (12,57). The pustules typically resolve in 4-10 days with a characteristic pinpoint desquamation.

Haematological abnormalities that may accompany the skin changes are leukocytosis, neutrophilia, and less commonly, eosinophilia (59). Involvement of the bone marrow may result in agranulocytosis. Systemic features are rarer than with other SCARs though pulmonary, renal and hepatic involvement have been described (57). The rate of mortality for patients with AGEP is less than 5% (12). Mortality in AGEP is strongly associated with multiple comorbidities and secondary infection.

3.2 Pathogenesis of AGEP :

The drug-specific T lymphocytes migrate to the epidermis and induce keratinocyte apoptosis, mediated by Fas ligand, perforin and granzyme B, in a manner similar to the pathogenesis of SJS/TEN. Enhanced expression of IL-8 and IL-3 stimulates neutrophil migration, resulting in the formation of the characteristic pustules.

More than 90% cases of AGEP are drug induced. The drugs commonly implicated in AGEP are antimicrobial agents such as penicillins, macrolides and quinolones, antimalarials, carbamazepine, paracetamol and terbinafine (12,57). The incidence of AGEP in patients on antiepileptics, sulfonamides and allopurinol is lower than the other SCARs (58).

3.3 Management of AGEP :

The diagnosis in AGEP is clinical. Roujeau et al (59) proposed the criteria for identifying potential cases of AGEP. A more elaborate scoring system was proposed by Sideroff et al (57) **(Annexure 7).** Acute generalised exanthematous pustulosis is generally amenable to treatment with topical corticosteroids and oral antihistamines. Severe manifestations may prompt the usage of short course systemic steroids.

IV.ORDINARY DRUG RELATED SKIN REACTIONS

There are several non-severe cutaneous adverse drug reactions ranging from pigmentation to urticaria. The common immunologically mediated non severe cutaneous adverse drug reactions are : maculopapular exanthema, erythema multiforme major and fixed drug eruption.

1.ERYTHEMA MULTIFORME MAJOR

Historically, erythema multiforme was believed to be a spectrum of disorders, namely, EM major, EM minor, SJS and TEN (19). But now, it is considered as a distinct hypersensitive response to triggers like drugs and infections (20). Erythema multiforme is characterised by a symmetrically distributed polymorphous rash with a preponderance for distal extremities (60). The classical phenotype comprises of target lesions (erythema iris) which may progress to epidermal detachment or oedematous papules (erythema papulatum), and mucosal involvement. There may be associated epidermal detachment EM minor does not affect more than one mucosa, whereas EM major may affect two or more mucosae

(61,62). More than half the reported cases occur secondary to herpes simplex virus infections. The drugs commonly implicated in EM major are barbiturates, phenytoin, NSAIDs, sulfonamides and penicillins (60).

2.MACULOPAPULAR EXANTHEMA

Maculopapular exanthema are characterized by an erythematous maculopapular eruption which occurs within 15 days of ingestion of a drug (63). This may be associated with fever, but the general condition of the patient is preserved. Peripheral eosinophilia may be seen in the range of 700 – 1000 cells/ml.

3.FIXED DRUG ERUPTION

Fixed drug eruptions usually manifest within 21 days of ingestion of the offending drug. They present as a limited number of erythematous plaques, which resolve with often long lasting pigmentation. There may be vesiculation overlying these plaques. A rechallenge with the drug typically produces recurrence of lesions at the same site (63,64).

V.DIAGNOSTIC CONSIDERATIONS IN CADRs

There are no conclusive diagnostic tests for CADRs. However, various molecular, serological and immunohistochemical aids have been developed for accurate diagnosis and prognostication of cutaneous adverse reactions.

1. HISTOPATHOLOGY :

1.1 SJS/TEN - Histopathological examination of the initial skin lesions of SJS/TEN show necrotic keratinocytes in the stratum basale with basal membrane vacoulization. Lymphocytic inflammatory infiltrate is accompanied by dermal eosinophilia. The later stages are characterized by subepidermal blistering. However, it has been reported that spongiosis, dermal oedema and eosinophilia are more common in SJS than in TEN (63).

1.2 DRESS - In DRESS syndrome there is a predominantly perivascular lymphocytic infiltrate with a small fraction of cases showing pustules (63). The infiltrate may also be in a lichenoid or interstitial pattern (63). Spongiosis and exocytosis are frequently observed, but basal cell vacuolization and eosinophilia are less common (63,65).

1.3 AGEP - Intracorneal, subcorneal and/ or intraepidermal pustules are seen in AGEP, with oedema of the papillary dermis which is often marked. There is a perivascular infiltrate composed mainly of neutrophils, and exocytosis of eosinophils. Vasculitis with few necrotic keratinocytes may be seen with an absence of epidermal detachment (57–59). These findings suggest a passive extrusion of neutrophils from the blood vessels in the upper dermis, which is then eliminated through the epidermis (66).

1.4 MPE - Maculopapular exanthema display a combined pattern of spongiosis, dermal edema with eosinophilia and perivascular infiltrate. Interface dermatitis and basal cell vacuolization are usually less intense in MPE(63,67,68).

1.5 FDE - The most common histopathological features noted in FDE are necrotic keratinocytes and basal cell vacuolization (63).

2. IMMUNOHISTOCHEMISTRY

Immunohistochemical studies on skin biopsies by Chung et al demonstrated intense staining of granulysin around the detached epidermis in SJS/TEN, as opposed to a weak staining in MPE (30). In the skin samples of healthy controls, granulysin was undetectable. This finding was reproduced by Weinborn et al. They additionally showed that there is an intense staining of granulysin in the inflammatory infiltrate in DRESS (63). Shlapbach et al studied tissue samples of patients with MPE, AGEP, FDE and TEN and showed that there is granulysin expression by CTLs and NKp46+ cells but with a varying intensity. The infiltration of NKp46+ cells was particularly high at the dermo-epidermal junction in TEN (69).

In yet another study by Cho et al, the expression of granulysin in tissue and serum were found to be paramount for distinguishing between generalized bullous FDE and SJS/TEN (70). There is also a higher expression of granulysin and perforin per CD8+ T cell in SJS/TEN than in EMM (71). Thus immunohistochemical studies on granulysin expression in tissue can be used to differentiate between phenotypically similar CADRs such as SJS and EMM, SJS/TEN and GBFDE and DRESS and MPE in the early phase of illness.

3. SKIN TESTS

3.1 Patch test :

A diluted formulation of the drug is topically applied to the skin and observed for a cutaneous response after a specified period of time. The drug-primed T cells will act as cytotoxic effector cells and recruit inflammatory mediators to the site, thus causing a localized reaction. The sensitivity and

specificity of the test cannot be accurately determined due to the lack of any standard tests against which it can be compared.

The test results are also dependent on a number of factors such as the type of drug being tested, its concentration and the vehicle used. For instance, the test is most reliable for antiepileptic agents such as phenytoin and carbamazepine. It must be ideally planned 2-6 months after the symptoms of the acute reaction subside. A positive test is highly predictive of a cutaneous adverse reaction but a negative test does not exclude its possibility. It is particularly safe and useful for identifying the drug responsible in AGEP and FDE (72).

3.2 Prick test

Prick test is performed on the volar aspect of the forearm with the drug and the excipient. The test is considered to be positive when a wheal of diameter > 3 mm and more than that of a control with normal saline is seen at the site after 20 minutes (73).

3.3 Intradermal test

Intradermal testing is contraindicated in patients with a history of EMM, SJS or TEN. Serial dilutions of the suspected drug is injected intradermally and observed for a wheal and flare response.

4. MOLECULAR TESTS

4.1 **Lymphocyte transformation test**

The lymphocyte transformation test is an in vitro procedure which measures the H-thymidine uptake by dividing T cells and is therefore an indirect measure of the activation of T cells in response to a

particular drug. The test is safe, reproducible, can simultaneously assess the T cell response to multiple drugs and can detect drug reactions via different immune-pathologic mechanisms. The sensitivity of the test is 60-70% and specificity is 85%. The sensitivity also depends on appropriately timing the test, i.e, 5-8 weeks after the onset of DRESS/DIHS. In contrast, the test can be done 1 week after the acute phase in SJS/TEN and maculopapular exanthema (74).

4.2 Macrophage migration inhibition factor (MIF) test

Macrophage migration inhibition factor is a chemokine released by antigen-sensitized T cells and is therefore, a reflection of cell-mediated immunity and delayed hypersensitivity (72,75,76). In MIF test, the patient's lymphocytes and guinea pig macrophages are incubated in serial dilutions of the suspected drug. The migration of macrophages in the presence and absence of the drug is used to calculate a migration index. A migration index of < 0.8 is considered as a positive test (76). The sensitivity and specificity for this test are 57% and 96% respectively (77).

4.3 Mast cell degranulation (MCD) test

This test measures the histamine released by mast cells after incubating with the suspected drug. It is a useful tool in evaluating type I hypersensitivity related CADRs (75). The sensitivity and specificity of this test have not been elucidated.

The other in-vitro tests that have been used in identifying the causative drugs in CADRs include lymphocyte toxicity assay, radioallergosorbent test (RAST) and interferon-gamma release test (75).

5. DRUG RECHALLENGE TEST

These are in vivo tests that comprises of re-introducing diluted forms of the suspected drugs in a serial manner. They include :

5.1 Oral provocation test ;

5.2 Substitution test.

Rechallenging a CADR patient with the suspected drug involves considerable risk and is undertaken in a controlled setting. It is contraindicated in patients with a history of SCARs.

6. SEROLOGICAL TESTS

In recent times, granulysin has gained popularity as an easily measurable biochemical marker in tissue or serum of patients with CADRs. Another biochemical marker, High Mobility Group Box 1 Protein (HMGB1) is under study for its role in the pathogenesis of SJS/TEN.

VI. ROLE OF GRANULYSIN IN CUTANEOUS ADVERSE REACTIONS

Severe cutaneous adverse reactions such as SJS/TEN and DRESS may present in the early stages with subtle skin lesions that resemble maculopapular and viral exanthema (4,78,79). In this setting, serological markers that can help in identifying the patients likely to progress to SCARs would help in initiating appropriate treatment, thereby reducing the rate of mortality.

1.1 Biochemical aspects of granulysin :

Granulysin is a multifunctional, cationic cytolytic granule protein released by human CTLs (CD8+ T cells) and natural killer (NK) cells and has broad antimicrobial and tumoricidal properties. It is also a chemoattractant and has pro-inflammatory properties. It belongs to the family of saposin-like lipid binding proteins and has two forms – a 15 kDa precursor and a 9 kDa effector form. The precursor form is constitutively secreted, can be measured in the serum and may reflect the host cell immunity (2). On the other hand, the 9 kDa form is released by a calcium dependent granule exocytosis pathway and not detected in the serum.

The 9 kDa form has cytolytic activity against microbes and tumors, whereas the 15 kDa form is elevated in acute viral infections and GVHD. The 15 kDa form is generally believed to be non-cytotoxic. However, Chung et al showed that at very high concentrations, the 15 kDa granulysin possesses a potent cytotoxicity similar to that of the 9 kDa form. Injecting purified 15 kDa granulysin into mouse skin caused epidermal and dermal necrosis similar to that seen in SJS/TEN (30).

1.2 Granulysin and immunity :

Granulysin has been shown to be elevated in a number of conditions such as acute viral infections, malignancy, transplantation, and cutaneous diseases such as psoriasis (80), acne (81), lichen planus (82) and folliculitis (83). It has also been identified as a useful marker for GVHD in allogenic stem cell transplant as its serum levels correlate with the clinical severity (84). Granulysin has a longer half- life as compared to other cytokines, and hence, it can be a useful marker of in-vivo cell mediated cytotoxic immune responses.

1.3 Granulysin in the pathogenesis of SJS/TEN :

It has been established that CTLs and NK cells infiltrate the skin in SJS/TEN. But the disseminated keratinocyte apoptosis in SJS/TEN is out of proportion to this infiltration of inflammatory cells. It was thus suggested that there may be soluble mediators which are responsible for this massive keratinocyte cell death. Chung et al showed that the blister cells in SJS/TEN primarily comprises of CD8+ CTLs and CD56+ NKT cells, and that both the cells and blister fluid are cytotoxic. They measured secretory granulysin (15 kDa form) in the blister fluid and found it to be the most highly expressed cytotoxic molecule, responsible for the disseminated keratinocyte apoptosis that characterizes the syndrome. The 9 kDa form of granulysin was not detectable. Granulysin titers were 2-4 fold higher than perforin, granzyme B and Fas ligand, and depleting the granulysin in the blister fluid reduced the cytotoxicity.

Chung et al proposed that specific targeted therapy against granulysin may be useful in treatment of SJS/TEN.

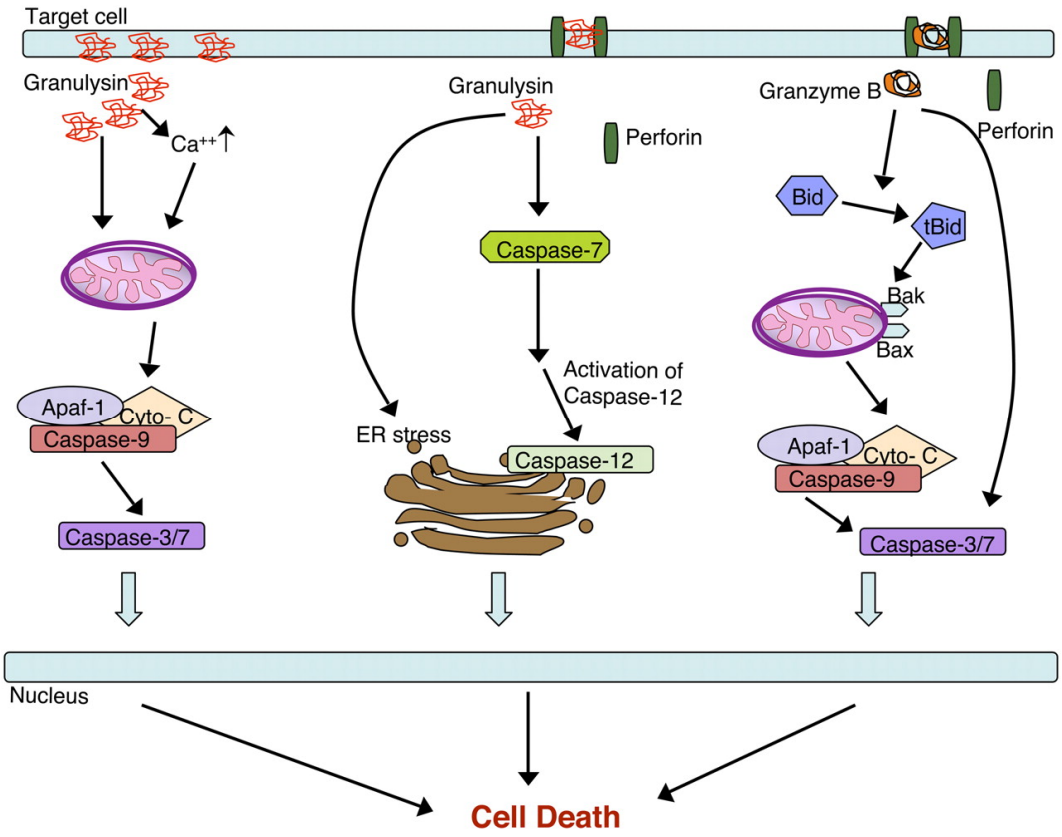


Figure 3 – Mechanism of cell apoptosis by granulysin

Adapted from saini et al (85)

Fujita et al recently showed that serum granulysin is elevated 2-4 days before the development of mucocutaneous lesions of SJS/TEN (86). This makes it a useful marker to distinguish between early stage-SJS/TEN and ordinary drug induced exanthemas, especially if the test is done 2-4 days before the development of blisters. They demonstrated that granulysin concentration in the serum falls rapidly within 5 days after the development of mucocutaneous bullae and erosions. They also developed a rapid immunochromatographic assay which correlates well with the sandwich ELISA method for the detection of serum granulysin. This test has 80% sensitivity and 95.8% specificity.

1.4 Other biochemical markers :

Another biochemical marker called High Mobility Group Box 1 Protein (HMGB1) has been found to be elevated in SJS/TEN(87). It is a 30 kDa non-histone nuclear protein and acts as a nuclear transcription regulator in the cells. It also activates the inflammatory cascade and thus can contribute to the pathogenesis of blistering in SJS/TEN. The sensitivity of HMBG1 assay is only 45.5%, but the advantage is that the HMBG1 levels are elevated for a longer time as compared to serum granulysin which normalizes within a few days of onset of bullae (87).

In this proposed study, we intent to describe the clinical profile of patients with adverse reactions, measure the serum granulysin levels in CADRs and describe its correlation with the severity of the illness. Even though serum granulysin in few subsets of SCARs have been studied previously, to the best of our knowledge, there are no studies which have included all SCARs and compared the same with healthy controls and maculopapular exanthema.

MATERIALS AND METHODS

Study design: This study was performed as a single center, prospective, case-control study on patients with cutaneous adverse drug reactions.

Study setting: The study was conducted at Christian Medical College, Vellore, Tamil Nadu. It is a tertiary care, 2858 bedded hospital, with an average out-patient attendance of 7000 per day. The Department of Dermatology, Venereology and Leprosy had an annual out-patient registration of 45839

in the year 2016 under Unit II. The study subjects were recruited from among the patients attending the Dermatology Unit II OPD and those referred from other departments as in-patient consultations.

Study subjects : Adult patients with a suspected drug induced rash were eligible for inclusion in the study.

Inclusion criteria :

i) Patients with drug induced maculopapular exanthema, erythema multiforme major, generalised fixed drug eruption and severe cutaneous adverse drug reactions (SJS/TEN, DRESS,AGEP, drug induced erythroderma)

ii) Patients above 18 years of age and who were willing to participate in the study.

Exclusion criteria :

(i) Patients less than 18 years of age.

(ii) Patients with viral exanthemas

(iii) Patients with a history of malignancy/ transplant/ tuberculosis/ past or present history of inflammatory skin diseases.

(iv) Patients not willing for participation in the study.

For the purposes of comparison, 20 age matched controls for SCARs were recruited.

Inclusion criteria for controls :

(i) Healthy volunteers over 18 years of age.

Exclusion criteria for controls:

(i) History of any preceding inflammatory skin disease/ malignancy/ tuberculosis/ transplant.

Period of recruitment : Study subjects were recruited from November 2015 to May 2017 (19 months).

Recruitment :

All patients with suspected drug induced exanthemas and severe cutaneous adverse reactions (based on clinical and biochemical examination) fulfilling the inclusion criteria and seen in Dermatology unit II OPD or as in-patient consultations were included in the study. Naranjo assessment (**Annexure 5**) was done

for each patient at the time of first contact of the patient by the principal investigator. An informed consent

was obtained from all the patients and controls (**Annexure 3**). Among the in-patients recruited for the study, if the patient was indisposed due to the illness to provide consent, it was obtained from the patient's attendant. The nature of the study and the investigation being done were explained to the patients

and their attendants.

Data collection : After obtaining the consent of the patient/ patient's attendant, the required details of the patient, including demographic details, history of the illness, clinical examination findings and relevant investigations were documented in a clinical research form **(Annexure 4).**

Demographic details : The name, age, gender, occupation, hospital number and contact details of the patient were documented.

History : A detailed history of the drugs ingested and the symptoms experienced by the patients were taken. These included :

- Latent period – the duration between the ingestion of the drug and appearance of the first symptom
- The number of days since the onset of the first symptom of the adverse drug reaction
- The duration of the rash
- Associated features like itching, burning sensation, mucosal involvement, swelling of face, hands and feet
- Systemic symptoms such as fever, jaundice, cough, arthralgia and breathlessness \
- Drug details such as the possible causative drugs and their indication, duration of drug intake, concurrent drugs used
- History of previous drug allergies and the type of previous CADR

Clinical examination : Vital signs comprising temperature, pulse rate, blood pressure and respiratory rate were noted. A thorough lymph node examination, including the number of sites were documented. A systemic examination was also performed on all the patients.

Cutaneous examination : The following clinical parameters were described in the patients :

- The presence of facial oedema
- Morphology of the skin lesions - blanching erythema, macular, papular, maculopapular, purpuric, targetoid, vesiculobullous, pustular or erosions
- The extent of the rash in terms of body surface area (BSA) as calculated by the "Rule of Nine"
- The number of mucosae involved, along with a description of the mucosal lesions
- Palmoplantar involvement
- Associated nail or hair changes

Photographs of the relevant skin lesions were taken. In addition, SCORTEN score was calculated in SJS/TEN patients.

Investigations : Routine haematological evaluation included counts, platelets and haemoglobin, liver and renal function tests, and HIV ELISA. An electrocardiogram, chest radiograph and urine eosinophil count were also done in patients with suspected visceral involvement.

Histopathological examination : Skin biopsy was performed on a case to case basis to rule out differentials like viral exanthems and connective tissue disorders. The biopsy was performed from the most representative skin lesion at the first assessment by the principal investigator. A skin punch of size 4 to 6 millimeters was used to do the biopsy and the specimen was transported to the department of Pathology in the routine skin fixative solution. The specimen was processed and then stained with eosin and hematoxylin stain. The slides were examined under light microscopy in the pathology laboratory by a senior pathologist. The following features in the specimens were noted – orthokeratosis**,** parakeratosis, necrotic keratinocytes, spongiosis, focal basal cell vacuolization, the type of inflammatory infiltrate and lymphocytic exocytosis.

Diagnosis :

The final diagnosis was made by the principal investigator and the guide on each included case after reviewing the history, clinical features, photographs and investigations.

All CADRs were further classified as :

- Maculopapular exanthema
- Severe cutaneous adverse reactions
- Erythema multiforme major
- Generalised fixed drug eruption

Severe cutaneous adverse reactions comprised of SJS/TEN spectrum, DRESS syndrome, drug induced erythroderma and AGEP.

The diagnostic criteria used for each of these were as follows :

DRESS syndrome : RegiSCAR criteria and RegiSCAR scoring were used to define DRESS syndrome.

RegiSCAR criteria comprises of the following essential criteria :

1. Hospitalization

2. Reaction suspected to be drug related

3. Acute skin rash

In addition, the patient has to have at least 3 of the following asterisked criteria.

4. Fever *

5. Enlarged lymph nodes *

6. Involvement of at least one internal organ*

7. Haematological abnormalities *

Each of the parameters in RegiSCAR criteria were defined as follows :

1) **Rash** refers to any skin eruption which had an erythematous component and /or macular/ papular component.

2) **Lymphadenopathy** was present if at least 2 non-contiguous sites were involved and the size of lymph nodes was more than 1 cm.

3) Fever was defined as a temperature above 100.4°F (38°C).

4) Transaminitis : Liver involvement was defined by the serum level of alanine aminotransferase or aspartate transferase greater than twice the upper limit of the normal values on at least one occasion (>80U/L)

5) Kidney involvement: Creatinine was considered abnormal if the serum level was more than 1.6 mg %

6) **Blood count abnormalities**:

- Absolute lymphocytes above 5000 /cubic mm (lymphocytosis) or below 1500/cubic mm (lymphopenia).
- Absolute eosinophils above 700/ cubic mm or >10% was considered to be peripheral eosinophilia.
- Platelets below 100,000/ cubic mm was considered to be thrombocytopenia.

Further, RegiSCAR scoring was used to classify patients as no case, possible DRESS, probable DRESS or definite DRESS.

RegiSCAR scoring for DRESS syndrome is outlined below.

Table 4 - RegiSCAR scoring

SCORE	-1	0	1	2
Fever > 38.5°C	No/U	yes		
Enlarged lymph nodes		No/U	yes	
Eosinophilia Eosinophils Eosinophils if TC < 4000/ml		No/U	 700/ml-1499/ml 10 - 19.9%	 >1500/ml >20%

Atypical lymphocytes		No/U	yes	
Skin involvement Extent of rash (% BSA) Skin rash suggestive of DRESS Biopsy suggestive of DRESS	 No No	 No/U U Yes/U	 >50% yes	
Organ involvement Liver Kidney Lung Muscle/heart Pancreas Other organs Resolution >15 days	 No/U	 No/U No/U No/U No/U No/U No/U Yes	 Yes Yes Yes Yes Yes yes	
Evaluation of other potential causes ANA Blood culture Serology of HAV/HBV/HCV/chlamydia/mycoplasma If none positive and 3 or more of the above negative		 yes		

U – unknown/ unclassifiable

(Final score <2 - no case, 2 to 3 – possible case, 4 to 5 – probable case, >5 – definite case)

Maculopapular exanthema : The cases with an acute cutaneous eruption, not fulfilling the RegiSCAR criteria were classified as maculopapular exanthemas.

SJS-TEN spectrum : The diagnosis was made on the basis of characteristic mucocutaneous findings, drug history and systemic symptomatology. Patients with purpuric, targetoid or atypical target lesions, vesiculobullous lesions or erosions with involvement of ≥ 2 mucosae, with a temporal relationship to a drug known to produce a severe cutaneous drug reaction were included in this group. Patients were further subclassified as SJS (BSA <10%), SJS-TEN overlap (BSA 10-30%) or TEN (BSA>30%) (88).

AGEP : Roujeau criteria (59), as outlined below, was used for the diagnosis of AGEP.

1. Several dozens of small, mostly non follicular pustules arising on a widespread oedematous erythema ;
2. Characteristic histopathological features – spongiform intraepidermal and/or subcorneal pustules, edema or papillary dermis, vasculitis, tissue eosinophilia and necrotic keratinocytes ;
3. Fever more than 38 degree Celsius ;
4. Blood neutrophil count more than 7 x 10^9/ L (7000/ml) ;
5. Acute evolution with spontaneous resolution of pustules within 15 days.

Additionally, the AGEP validation score of the EuroSCAR study group (57) was used to score patients with AGEP **(Annexure 7).**

Erythema multiforme major : Patients who presented with predominantly acrally distributed target lesions or raised oedematous papules with involvement of one or more mucous membranes were clinically diagnosed to have EMM (89).

Fixed Drug Eruption : A diagnosis of FDE was made in patients with dusky erythematous macules or plaques with a tendency to resolve with often long lasting post inflammatory pigmentation. Recurrence of lesions at the same sites on drug rechallenge and involvement of less than two mucosae were additional clues to diagnosis (90).

Controls : Twenty age-matched healthy volunteers over the age of 18 years were recruited as controls. One control was recruited for each case with a SCAR. The controls were inducted from patients attending the Dermatology OPD for minor skin complaints such as melasma, skin tags and xanthelasma palpebrum. Demographic details were entered in the proforma. Individuals with a history of previous skin disease/ malignancy/ transplant or any other recent illnesses were not included.

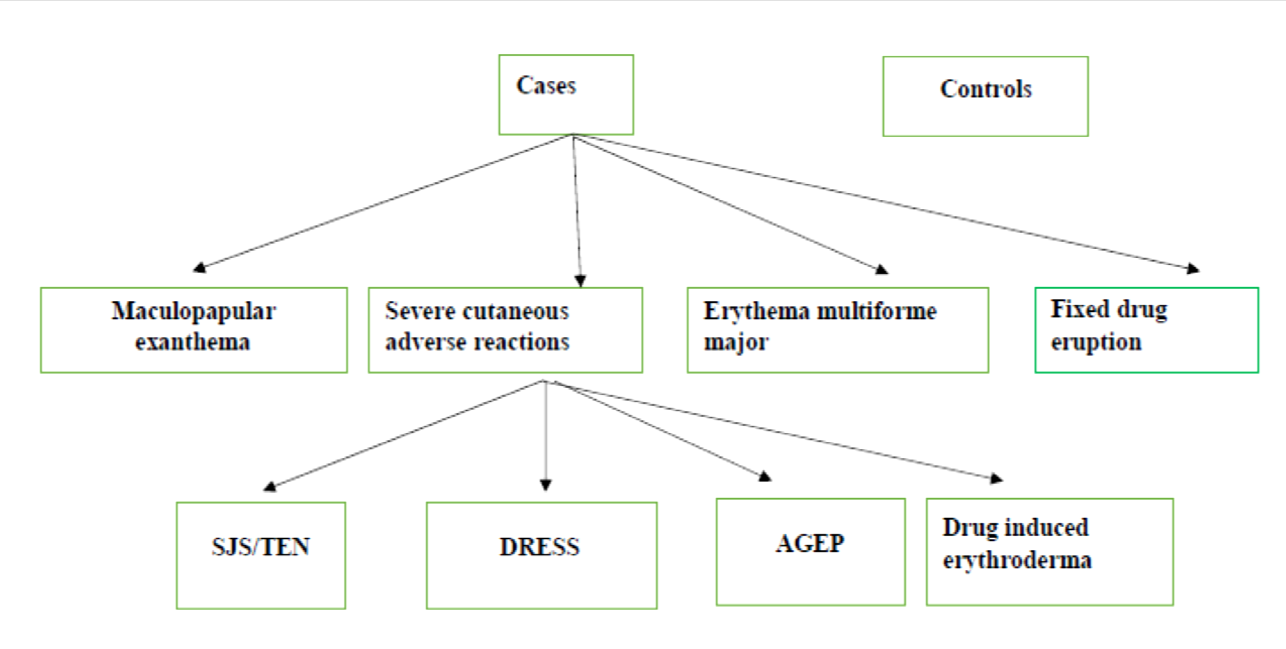


Figure 4 - Algorithm for classification of the study subjects

Measurement of serum granulysin : A blood sample was obtained for the measurement of serum granulysin for all the study patients at initial presentation. Samples were collected in EDTA tubes and transported to the Department of Clinical biochemistry at Christian Medical College, Vellore, by the principal investigator for all study subjects. The serum was frozen and stored at -70 degree Celsius in the laboratory till the time of the assay. The granulsyin concentrations of the serum samples were measured with Biovendor RD191327200R Human Granulysin ELISA, a sandwich-enzyme linked immunosorbent assay. The sensitivity of this test is 0.03ng/ml and there is no cross-reactivity with pro-saposin.

Standards (recombinant protein based) and samples were incubated in micro-titration wells pre-coated with polyclonal anti-human granulysin antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-human granulysin antibody was added and incubated with the captured granulysin for 60 minutes. Streptavidin-HRP conjugate was added after washing the wells. After 30 minutes of further incubation followed by washing, the remaining conjugate was allowed to react with the substrate solution, tetramethylbenzidine (TMB). Absorbance of the resulting yellow product was proportional to the concentration of granulysin in the sample. A standard curve was constructed by plotting absorbance values against granulysin concentrations of standards. The concentration of granulysin in the samples were determined using this standard curve. The test results were verified by a senior biochemist.

Statistical analysis :

Calculation of sample size :

Here the primary objective is to calculate the mean value of serum granulysin and its confidence interval. Previous studies have shown that the mean concentration ± standard deviation (SD) of serum granulysin among healthy individuals was 3.7 ± 3.2 ng/ml (2) and that among patients with drug exanthema was 3.5 ± 3.4 ng mL (3). The mean concentration ± standard deviation of severe drug reactions was 30.35 ± 9.91 ng/mL. Hence, the sample size for the three groups were calculated as follows :

With expected mean (SD) for SCARs group as 30(10), maculopapular exanthema as 10(3) and healthy controls as 10(3), the minimum required number of samples to be studied are n=16 on each group. The formula used is

Formula

$$n = \frac{2s_p^2\left[Z_{1-\alpha/2} + Z_{1-\beta}\right]^2}{\mu_d^2}$$

$$s_p^2 = \frac{s_1^2 + s_2^2}{2}$$

Where,

s_1^2 : Standard deviation in the first group

s_2^2 : Standard deviation in the second group

μ_d^2 : Mean difference between the samples

α : Significance level

1- β : Power

Here alpha level is taken as 1% and power as 90%.

Statistical methods :

The data entry was performed using Epidata software and analysis by using SPSS software. The independent sample t test was used for the comparison of two categories with normal variables. For variables without normal distribution, Mann- Whitney's U test was used. The association between 2 categorical variables was checked by the chi-square test and the graphical representation of the analysis was done with bar plot and error plot. The analysis was done by Ms.Tunny Sebastian, Department of Biostatistics, Christian Medical College, Vellore.

IRB approval : This study was approved by the Institutional Review Board of Christian Medical College, Vellore, bearing IRB Min number 9674 on 20/10/2015 and was funded by the Fluid research grant of the institution (**Annexure 1**).

RESULTS

Forty eight patients with cutaneous adverse drug reactions who fulfilled the inclusion criteria were recruited during the study period of November 2015 to May 2017 (19 months). Among this cohort, 22 patients had drug induced maculopapular exanthema, 21 patients had severe cutaneous drug eruptions, 4 patients had erythema multiforme major and 1 patient had generalised fixed drug eruption. Among the SCARs, we diagnosed 12 patients with SJS/TEN, 7 patients with DRESS syndrome and one patient each with drug induced erythroderma and AGEP. The seven patients who fulfilled the RegiSCAR criteria for DRESS were identified as possible (4 cases) and probable DRESS (3 cases) by RegiSCAR scoring. The patient with AGEP received a score of 11 on the AGEP validation score by EuroSCAR study group and was classified as a definite case.

Twenty age matched healthy volunteers were also included as controls.

1. DEMOGRAPHIC PROFILE :

1.1 Age :

Out of the 48 patients included in the study, 19 patients (40%) belonged to the age group of 18-40 years and 22 patients (46%) belonged to the group of 40-60 years. Only a minority (7/48, 14%) of the included patients were aged over 60 years. The age distribution of the study population is shown in **figure 5**. The mean age was 44.4 ± 14.6 years.

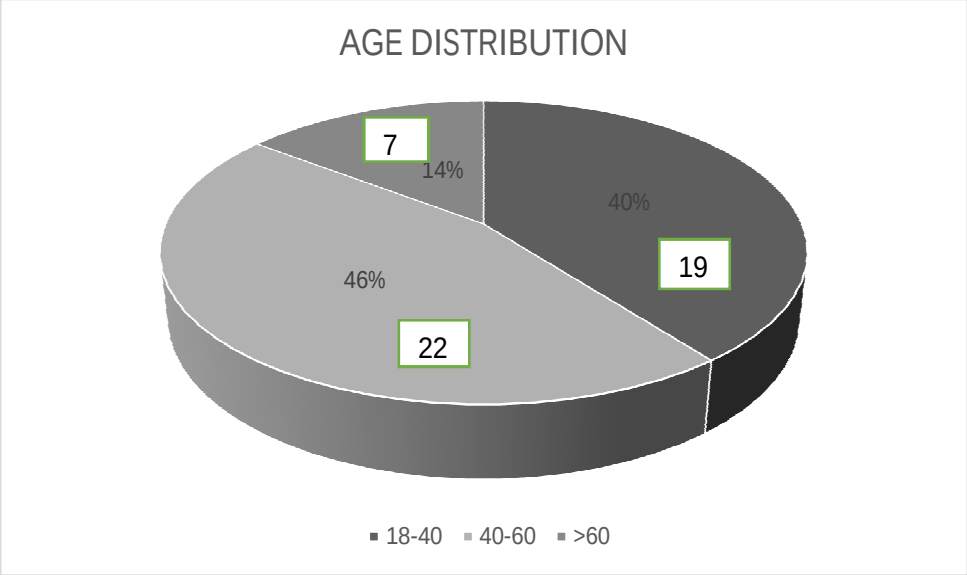


Figure 5 - Age distribution of patients with CADRs

1.2 Gender :

Seventy one percent (34/ 48) patients with CADRs were women as against 29% (14/48) men **(figure 6).** This shows a significant female preponderance with a male: female ratio of 0.41:1.

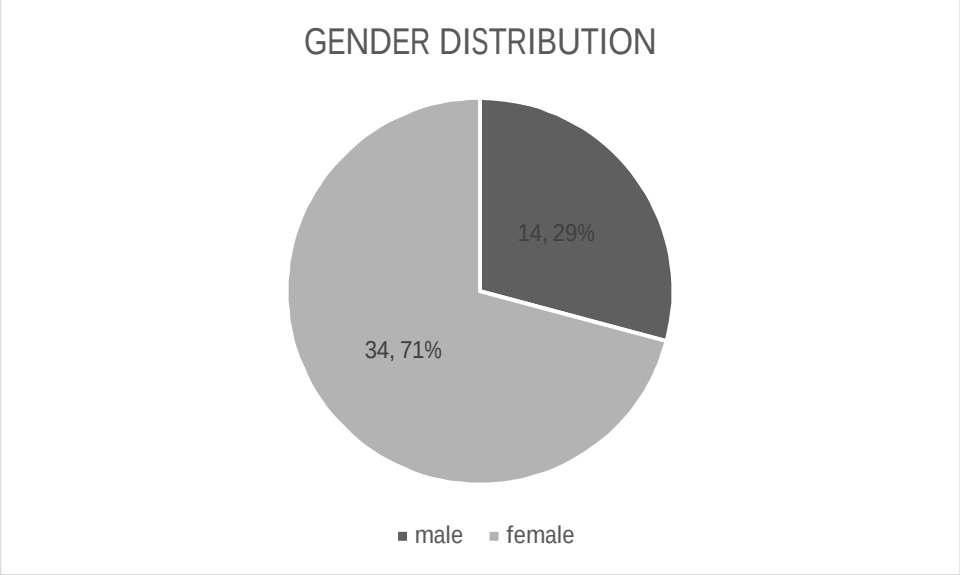


Figure 6 - Gender distribution of patients with CADRs

Both the MPE group and SCARs group showed a female predominance with 15/22 patients (68.18%) in the MPE group and 16/21 patients (76.19%) in the SCARs group comprising of women. Erythema multiforme major group showed equal number of males (2/4) and females (2/4). Only 1 female patient was present in the fixed drug eruption group. The gender distribution among the different types of CADRs is represented in **figure 7**.

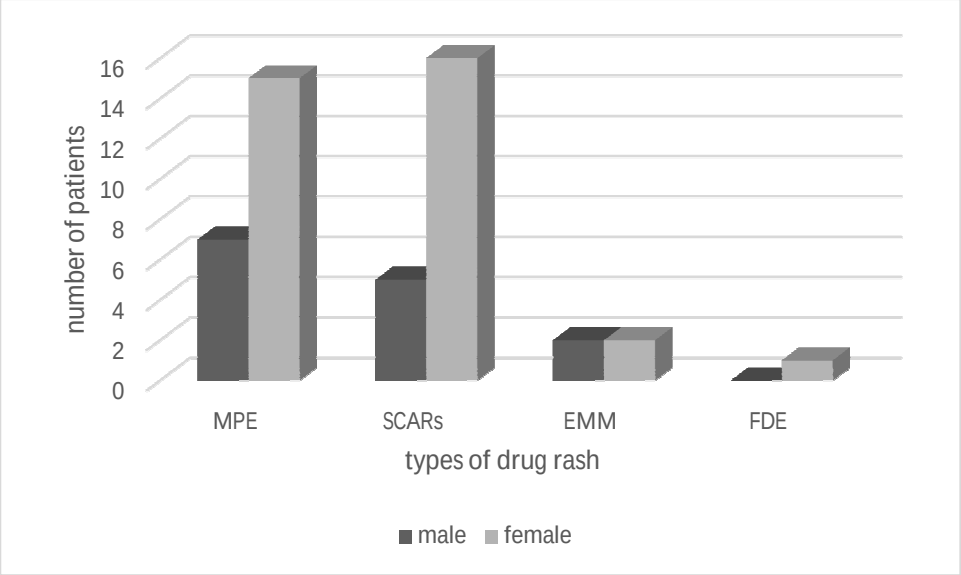


Figure 7 - Gender distribution of different types of CADRs

1.3 Place of origin

Majority of the patients were from Tamil Nadu (33/48, 68.75%), followed by Andhra Pradesh (7/48, 14.6%). Three patients each were from West Bengal and neighbouring countries like Bangladesh and Bhutan. One patient each hailed from Kerala and Orissa **(figure 8).**

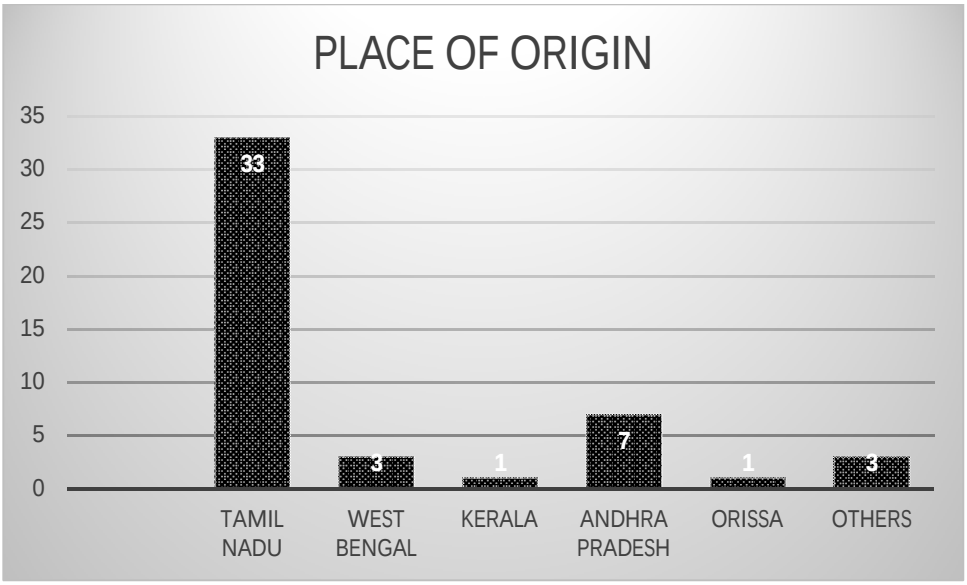


Figure 8 - Place of origin of patients with CADRs

2. CLINICAL PROFILE

2.1 Presenting symptoms :

The most common symptoms at presentation were fever with mucocutaneous involvement (19/48, 39.6%), followed by fever with skin involvement (17/48, 35.4%) and cutaneous involvement alone (12/48, 25%) **(figure 9).**

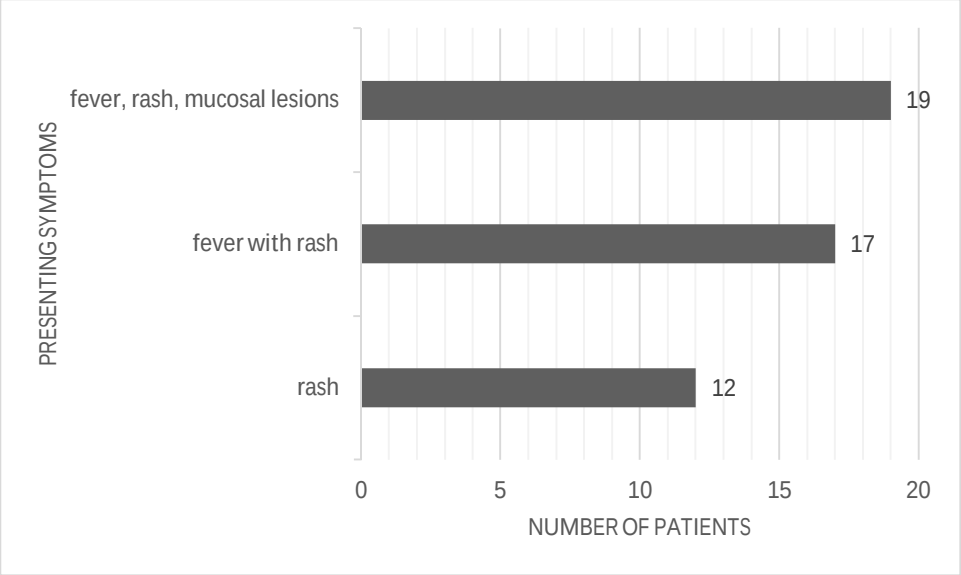


Figure 9 - Presenting symptoms of patients with CADRs

2.2 Latent period :

The time period between the onset of drug intake and first symptom is shown in **table 5**.

Table 5 – Latent period with the different CADRs

Type of CADR							
Parameter	AGEP	SJS/TEN	DRESS	Erythroderma	MPE	FDE	EMM
Mean ± SD (days)	1	12.5 ± 9.1	16.1 ± 10.3	27	8.4 ± 7.2	1	21 ± 27.4

The latent period in AGEP and FDE was 1 day, and in erythroderma, it was 27 days. Among the other CADRs, MPE had a shorter latent period (8.4 ± 7.2 days) as compared to SJS/TEN (12.5 ± 9.1 days) and DRESS (16.1 ± 10.3 days). The EMM group had a mean latent period of 21 ± 27.36 days.

2.3 Other symptoms in CADRs :

Besides fever and rash, the most common symptoms in the study patients were oedema of the face, hands or feet, followed by cough, dyspnea, jaundice and arthritis respectively. The frequency of these symptoms in CADRs is shown in **figure 10**.

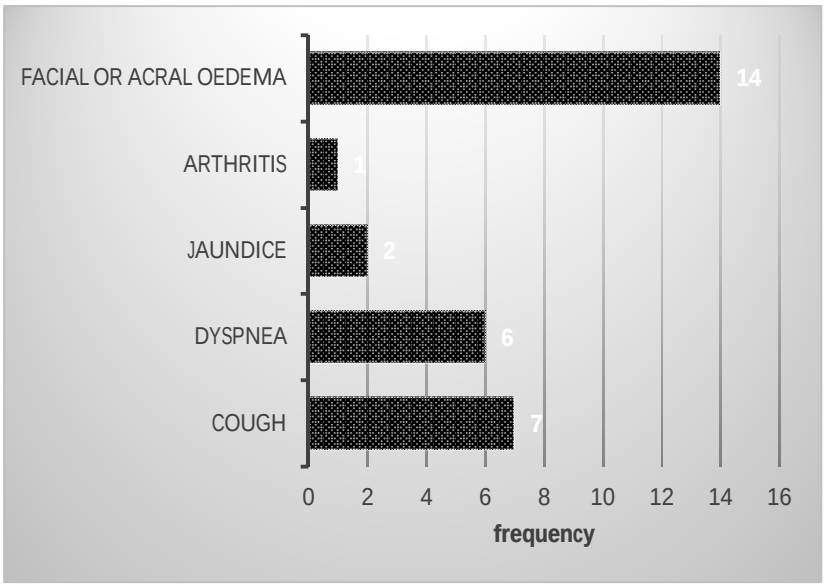


Figure 10 - Symptoms in patients with CADRs

In our study, we found that facial oedema was seen in 14 patients (29.2%) of the total patient population, (6 SCARs, 5 MPEs, 2 EMM and 1 FDE). In addition, a history of facial oedema was elicited in 3 patients (two patients with drug induced erythroderma and one patient with MPE). However, on examination, facial oedema had resolved in these patients. The prevalence of facial oedema in our study population is represented in **table 6.**

Table 6 - Prevalence of facial oedema among CADRs

CADR	Frequency	Percentage
MPE	5/22	22.7%
SCAR	6/21	28.57%
EMM	2/4	50%
FDE	1/1	100%

2.4 Day of presentation :

Fifty eight percent of patients with CADRs presented within 5 days of onset of complaints, whereas 27% presented between 6-10 days and 15% after 10 days **(figure 11)**. The median day of presentation in MPE was 4 days (IQR 2 – 7.75 days) and that in SCARs was 5 days (IQR 3 – 9.5 days).

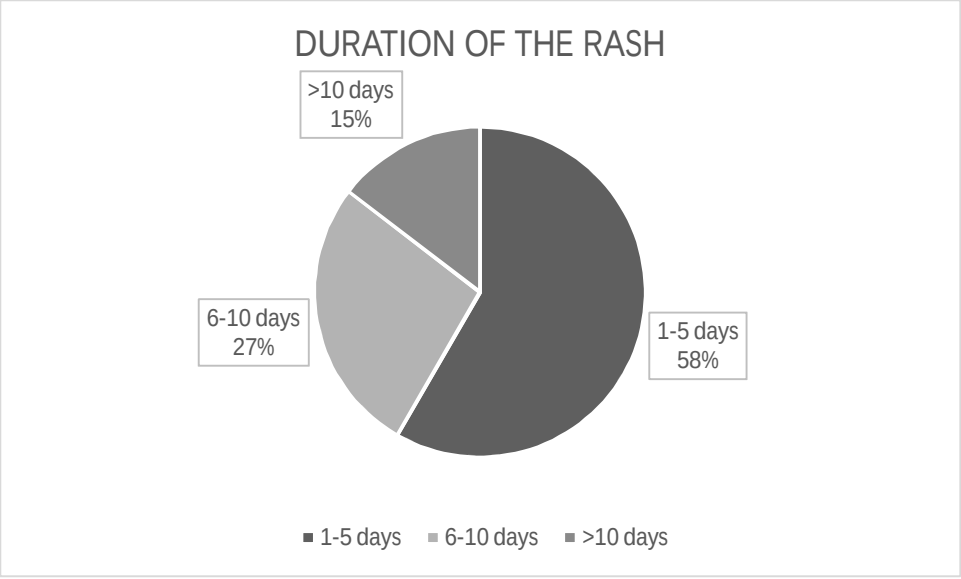


Figure 11 – Duration of onset of the rash

Majority of the men with CADRs (64%) presented within 5 days of onset of symptoms, 42.8% between 6-10 days, and 7% after 10 days of onset of symptoms. Among women, 55.9% presented early in the course of illness, i.e, within 5 days, 20.6% within 6-10 days and 17.6% after 10 days of onset of complaints **(Table 7)**.

Table 7 - Gender distribution vs day of onset of symptoms

Number of days since the onset of complaints	Male	Female
1-5 days	9 (64.3%)	19 (55.9%)
6-10 days	6 (42.8%)	7 (20.6%)
>10 days	1 (7%)	6 (17.6%)

2.5 Comorbidities :

Majority of the patients (n=45) had diseases other than the CADR. Diabetes was present in 10 patients (20.8%) and hypertension in 11 patients (22.9%).

2.6 Alcohol consumption : Only 4 patients (8.3%) gave history of alcohol consumption. All of these patients were males.

3. DRUG DETAILS :

3.1 Commonly implicated groups of drugs in CADRs :

The most common group of drugs implicated in CADRs were antiepileptic drugs (35.4%) like phenytoin and carbamazepine, followed by antibiotics (18.8%). In 10 patients (20.8%), the causative drug was suggested to be either antibiotics or others (such as proton pump inhibitors, diuretics, sulfa drugs, anti-tuberculous drugs) by Naranjo algorithm. There were 2 patients (4.2%) who were on both antibiotics and antiepileptic drugs and the causative drug could not be accurately determined. The frequency of different groups of causative drugs is represented in **table 8**.

Table 8 - Commonly implicated groups of drugs in CADRs

	Total frequency	MPE	SCARs	EMM
Antiepileptic agents	17 (35.4%)	4 (23.5%)	10 (58.8%)	3 (17.64%)
Antibiotics	9 (18.8%)	6 (66.67%)	2 (22.2%)	1 (11.1%)
Others	7 (14.6%)	5	2	
Antiepileptics/ Antibiotics	2 (4.2%)	1	1	
Antiepileptics/ others	2 (4.2%)		2	
Antibiotics/ others	10 (20.8%)	4	3	
Unknown	1 (2.1%)	1		

Antiepileptic agents were implicated as causative agents in 47.6% SCARs whereas antibiotics were the common culprit drug group (27.3%) among MPE. This table is represented in **figure 12**.

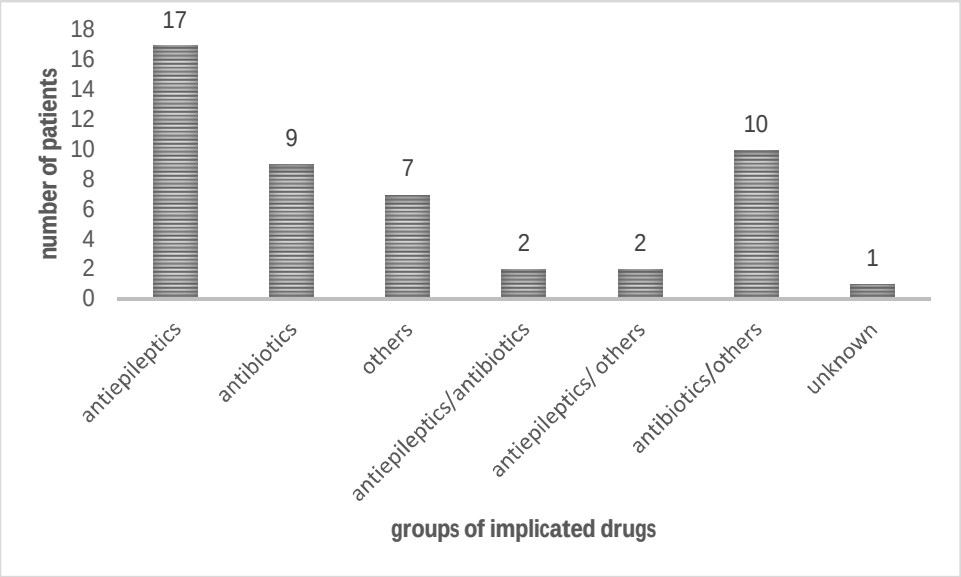


Figure 12 – Frequency of implicated drugs among patients with CADRs

We identified one patient with a suspected MPE to siddha medicines taken for cold and eczema. Another patient who was treated with carbamazepine and rituximab for neuromyelitis optica spectrum disorder developed SJS. A diagnosis of carbamazepine induced SJS was considered in view of the temporal relationship to the drug and the typical morphology of skin lesions. As the patient had also received Rituximab just prior to developing the skin lesions, by Naranjo causality assessment, rituximab induced SJS was also considered as a possibility. Another case was an MPE to rabies vaccine/ immunoglobulin.

3.2 Past history of drug hypersensitivity :

Five patients in the cohort had a past history of drug allergy. They had subsequently developed MPE (n=2), FDE (n=1), drug induced erythroderma (n=1) and AGEP (n=1). The patient with the generalised FDE had a past history of FDE to an unknown drug. The current episode also followed ingestion of an unknown drug over the counter. The patient with AGEP to penicillin reported a non-severe drug reaction previously to the same drug. The other 3 patients had a past history of drug hypersensitivity to drugs unrelated to the culprit drug in this study.

4. RASH CHARACTERISTICS

4.1 Morphology of the rash :

The morphology of the skin lesions seen in the CADRs were varied. The most common skin lesions seen in the study cases were blanching erythema and papular skin lesions (17/48, 35.4% each), closely followed by target or atypical target lesions and erosions (16/48, 33.3% each). Fourteen patients had vesiculobullous skin lesions at the time of initial presentation, comprising of 29% of the total number of cases. Purpuric lesions, macules and pustules were seen in much less frequency. The additional skin findings were : flexural accentuation of the rash (n=3), scaling and desquamation and crusting **(figure 13)**. In 6 patients, the papules had an infiltrated appearance, in keeping with the findings expected in DRESS.

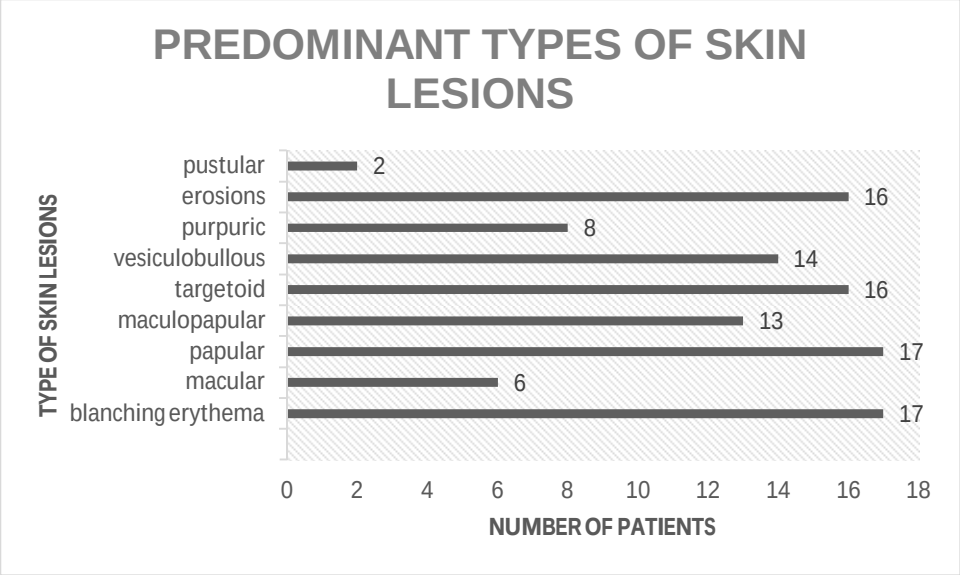


Figure 13 – Frequency of the different types of skin lesions in study patients

A patient with MPE to chlorpromazine had a photo-distributed rash with maculopapular skin lesions on the exposed areas of the face and upper limbs.

4.2 Body surface area involved in CADRs :

Half of the study patients (n=24, 50%) had involvement of more than 30% BSA, while 25% had 10-30% BSA and 25% had less than 10% BSA involved **(figure 14)**.

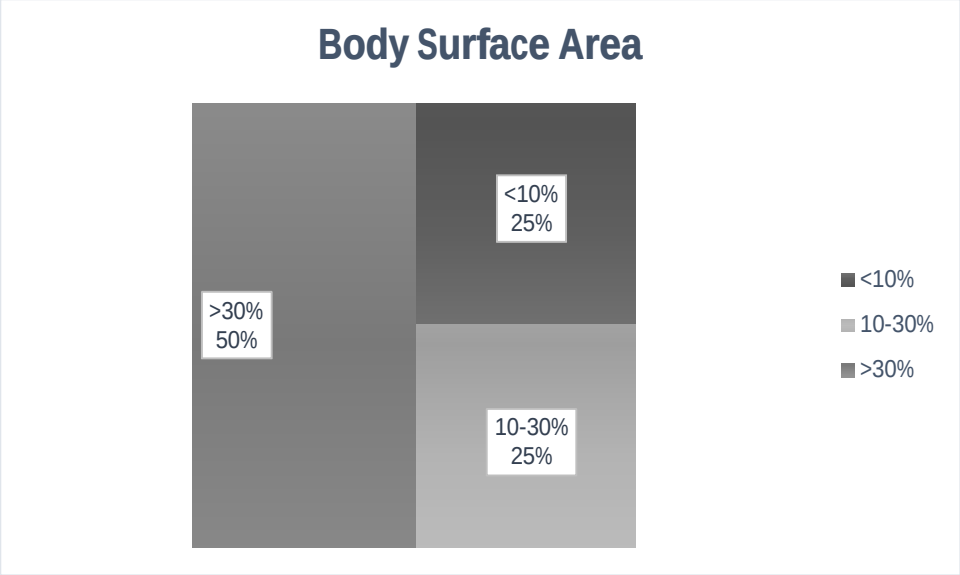


Figure 14 – Body surface area involved in the study population

The body surface area involvement in MPE and SCARs groups is shown in **table 9.**

Table 9 – Body surface area involved in MPE and SCARs

BSA	MPE	SCARs
<10%	4 (18.2%)	5 (23.8%)
10-30%	7 (31.8%)	4 (19.04%)
>30%	11 (50%)	12 (57.1%)
Total	n = 22	n = 21

This table shows that 50% MPE cases and 57% SCARs had extensive skin involvement of more than 30% body surface area. All 5 patients who had limited skin involvement in SCARs (<10%) were cases of SJS.

4.3 SCORTEN score for SJS/TEN :

SCORTEN score was calculated for all our patients with SJS/TEN at admission and is represented in **table 10.**

Table 10 – SCORTEN score for patients with SJS/TEN

SCORTEN score	**SJS**	**SJS-TEN overlap**	**TEN**
1	2	1	0
2	0	0	0
3	1	1	1
4	0	0	3
≥ 5	0	0	1

It can be seen that patients with TEN had a higher SCORTEN score than SJS and SJS-TEN overlap and thus, were at a higher risk of mortality.

4.4 RegiSCAR score :

RegiSCAR scoring was done for all patients with suspected DRESS. Based on this score, we stratified patients as having possible or probable DRESS and no case of DRESS (MPE). The mean RegiSCAR score for DRESS cases was 3.4 ± 0.97 and for MPE was -1.1 ± 1.7 (p value <0.001).

4.5 Palmoplantar involvement :

Palmoplantar involvement was seen in 15 patients (15/48, 31.25%), with palmar lesions (n=13) being slightly more common than plantar lesions (n=11). The commonest morphological forms noted were atypical target lesions, blanching erythema, petechiae, purpura, collapsed bullae and scaling. Palmoplantar involvement was more common in SCARs than in MPE (9/21, 42.85% vs 3/22,13.6%).

4.6 Facial involvement :

Facial lesions were seen in 26 patients, comprising 54.2% of the cases included in the study. The MPE group showed facial involvement in 10 out of the 22 patients (45.5%). The most common morphological types of rash noted on the face in patients with MPE were facial oedema, blanching erythema, papules, macules and desquamation. Few patients also had features like perioral and periorbital papules and erythema of the ears.

In the cohort of SJS/TEN, facial involvement was seen in 7 patients (58.3%). Atypical target lesions and facial oedema were the commonest findings, followed by purpura, vesiculobullous lesions and erosions. Majority of the patients with DRESS syndrome (n=3, 42.8%) had facial involvement, which consisted of facial oedema (n=2), erythema (n=1) and desquamation (n=2). Facial erythema was seen in the patient with drug induced erythroderma. Facial oedema was also seen in the patients with acute generalized exanthematous pustulosis and generalised fixed drug eruption. Two out of the four patients with erythema multiforme major had facial oedema. The distribution of facial rash in CADRs is shown in **figure 15.**

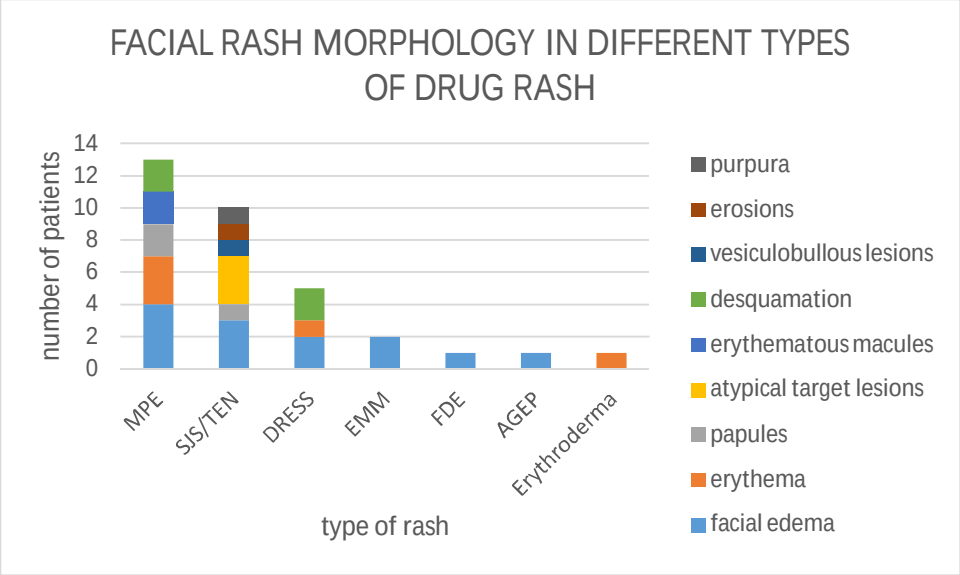


Figure 15 - Morphology of facial rash in CADRs

4.7 Mucosal involvement :

Conjunctival lesions were seen in12 patients (25% of the cases), oral lesions in 23 patients (47.9%) and genital involvement in 4 patients (8.3%). The frequency of at least one mucosal involvement was much higher in the SCARs group as compared to MPE group. Among the 22 patients with MPE, 3 patients had oral lesions (13.6%). One of these patients had cheilitis and another had discrete oral ulcers. A third patient had multiple oral ulcers which were probably secondary to neutropenia. Conjunctiva and genitalia were spared in MPE. All 4 patients with erythema multiforme major had oral mucosal lesions, 2 patients (50%) had conjunctival lesions and one patient (25%) had genital lesions. The distribution of mucosal lesions in CADRs is represented in **figure 16.**

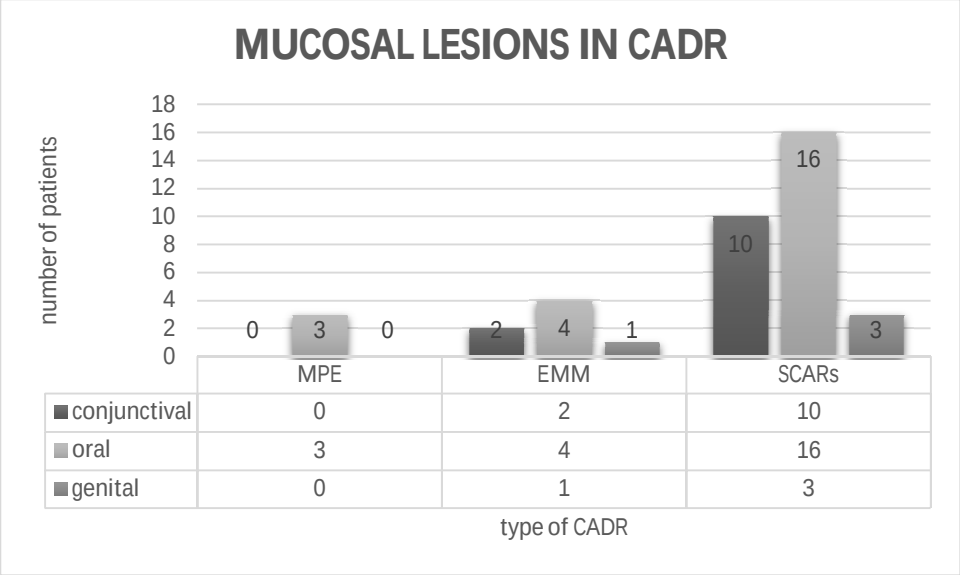


Figure 16 – Frequency of mucosal lesions in CADRs

5. SYSTEMIC INVOLVEMENT

The various laboratory parameters analyzed among the patients with CADRs showed haematological abnormalities and transaminitis to be the most frequent findings. Deranged renal function tests, pneumonitis and carditis were rarer.

5.1 Lymphadenopathy :

Significant lymph node enlargement was seen in 8 patients, with 4 patients having enlargement of 2 or more groups of lymph nodes. Only SCARs were associated with lymphadenopathy at more than two anatomical sites, whereas patients with MPE and generalized FDE had only involvement of a single group of nodes. The distribution of lymphadenopathy among CADRs is shown in **figure 17**.

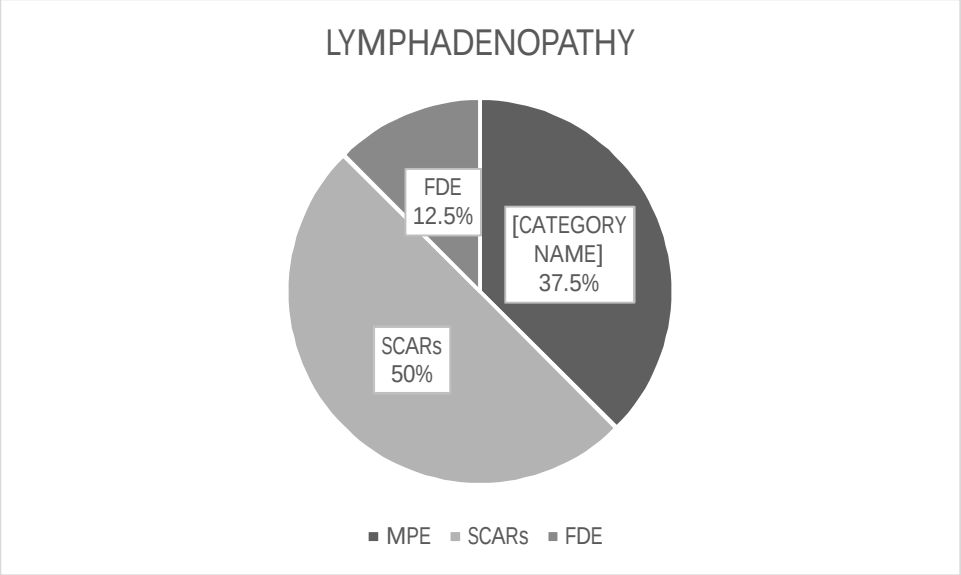


Figure 17 – Lymphadenopathy in patients with CADRs

5.2 Involvement of the respiratory system :

Six out of the 48 patients (12.5%) had respiratory symptoms like cough and dyspnea, while abnormal chest radiograph findings were seen in 9 patients (18.75%). Three of these patients belonged to the MPE group and 5 patients were from the SCARs group (all 5 patients had SJS/TEN). Among the MPE patients, one patient had developed dyspnea following a surgical debridement for necrotizing fasciitis and her chest X-ray showed bilateral heterogenous lung infiltrates, which was probably not related to the CADR.

Thus, respiratory findings were more common among SCARs as compared to MPE, but the difference was not statistically significant (p value 0.098).

5.3 Involvement of the gastrointestinal system :

Gastrointestinal involvement was present only in 2 patients on clinical examination. One patient had hepatomegaly, while the other had ascites and abdominal distension. Transaminitis was seen in one third of the patients with SCARs (n=7/21, 33.3%) and only in one patient with MPE **(table 11).** Thus, transaminitis was significantly more common among SCARs than MPE (p value 0.021).

5.4 Involvement of the renal system :

Raised creatinine was seen in 3 patients each in the MPE and SCARs groups. The diagnosis in the 3 SCARs patients was SJS/TEN. Out of the 3 MPE patients with raised creatinine, two suffered from chronic kidney disease and the third had multi-organ dysfunction.

5.5 Involvement of the cardiovascular system :

The patient with AGEP had cardiomegaly on chest radiograph. Electrocardiogram was not available for routine analysis among our study patients. Among the DRESS patients, ECG changes suggestive of mild ischemia was seen in one patient (n=1/7, 14.3%).

5.6 Peripheral eosinophilia in CADRs :

Eight patients included under MPE (36.4%) showed peripheral eosinophilia, with 4 patients having marked eosinophilia of >1000 eosinophils/ml. Thirty five percent of the patients with SCARs also showed eosinophilia **(figure 18)**.

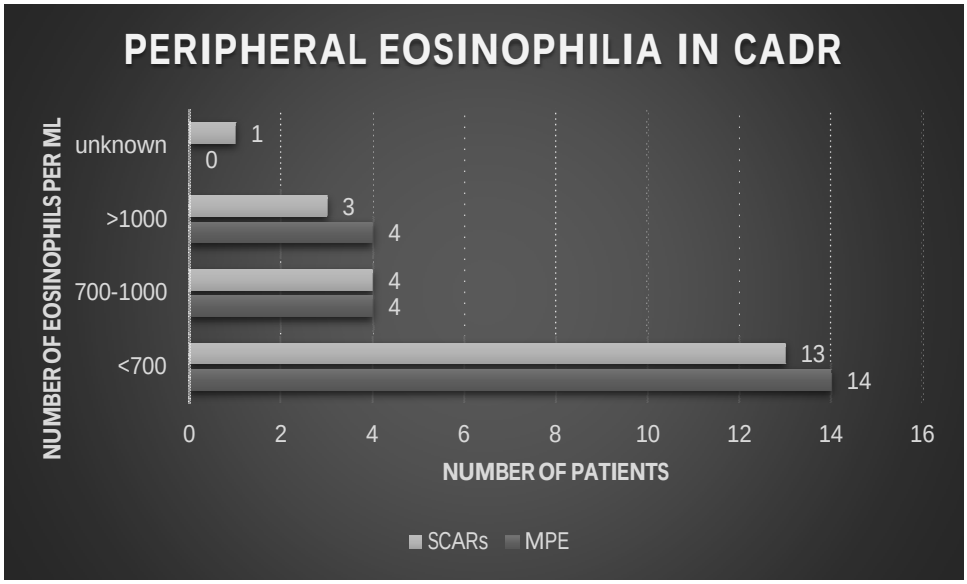


Figure 18 – Frequency of peripheral eosinophilia in CADRs

5.7 Other haematological parameters :

Lymphocytopenia was the most frequently encountered haematological abnormality and was present in 33 of the 48 patients (68.75%). Lymphocytosis was not seen in any of our patients. Thrombocytopenia was more common in severe cutaneous adverse reactions as compared to the maculopapular exanthemas (28.6% *vs* 9.1%).

The frequency of haematological, hepatic and renal abnormalities in CADRs is shown in **table 11**.

Table 11 - Abnormal laboratory parameters in CADRs

	MPE	SCARs	p value
Lymphocytosis	0	0	
Lymphocytopenia	13 (59.1%)	16 (80%)	0.232
Thrombocytopenia	2 (9.1%)	6 (28.6%)	0.132
Transaminitis	1 (4.5%)	7 (33.3%)	0.021
Raised creatinine	3 (13.6%)	3 (14.3%)	0.345
Urine eosinophils	3 (13.6%)	0	

6. HISTOPATHOLOGICAL ANALYSIS

Biopsy was not routinely done for all patients. It was done on a case to case basis to differentiate CADRs from connective tissue disorder or alternate diagnoses.

Out of the 48 patients included, 50% (n=24) underwent biopsy, of which 12 patients were eventually classified as MPE, 10 patients were SCARs and 2 were erythema multiforme major. The most common histopathological feature seen in our study patients was dermal eosinophilic infiltrate (83.3%). This was followed by lymphocytic exocytosis and necrotic keratinocytes in 66.67% each and spongiosis in 62.5%. Necrotic keratinocytes were seen in all the patients with SCARs whereas lymphocytic exocytosis and dermal eosinophils were seen in 90% patients with SCARs.

The frequency and percentage of the most common histopathological findings among CADRs is represented in **table 12.**

Table 12 - Frequency of histopathological features in CADRs

	Total frequency (n=24)	MPE (n=12)	SCARs (n=10)	p value
Spongiosis	15 (62.5%)	7 (58.3%)	7 (70%)	0.321
Necrotic keratinocytes	16 (66.67%)	5 (41.6%)	10 (100%)	0.01
Basal cell vacoulization	10 (41.6%)	3 (25%)	6 (60%)	0.192
Lymphocytic exocytosis	16 (66.67%)	6 (50%)	9 (90%)	0.045
Dermal eosinophils	20 (83.3%)	10 (83.3%)	9 (90%)	>0.99

Focal basal cell vacoulization was seen in 71.4% patients with DRESS (n=5) and 60% patients with SJS/TEN (n=7). Lymphocytic exocytosis and necrotic keratinocytes were seen in all the patients with DRESS and SJS/TEN, and was significantly higher in SCARs than MPE (p value 0.045 and 0.01 respectively). These findings were seen among 50% and 41.6% of MPE respectively. Spongiosis was seen in 58.3% patients with MPE (7 out of the 12 MPE patients). It was more common among the DRESS patients (100%, n=4, p value >0.99) but not statistically significant. Among patients with SJS/TEN, spongiosis was seen in 40% (2 out of 5 patients).

7. SERUM GRANULYSIN IN CADRs :

Serum granulysin was measured in all the cases included in the study by the Biovendor RD191327200R Human Granulysin ELISA, a sandwich-enzyme linked immunosorbent assay. In addition, serum granulysin was also assessed in 20 age matched controls.

The results of our study are outlined below.

7.1 Serum granulysin titer in CADRs :

The distribution of serum granulysin across the different types of drug rashes were skewed as seen in **figure 19.** Majority of the patients had serum granulysin values less than 1.00, except 2 patients in MPE group and one patient in SCARs group who had value >4.0. It was seen that the MPE patients with granulysin > 4 ng/ml (upper limit of detection by the test) had necrotizing soft tissue infections at the time of inclusion in the study. Hence they were excluded at the time of analysis of granulysin levels **(fig 19)**.

The median value of serum granulysin in the different CADRs were :

1. Maculopapular exanthema – 0.15 ng/ml (IQR 0.1075 – 0.2925)
2. Severe cutaneous adverse reactions – 0.26 ng/ml (IQR 0.15 – 0.5)
    a. SJS/TEN – 0.275 ng/ml
    b. DRESS – 0.24 ng/ml
    c. AGEP – 0.1 ng/ml
3. Drug induced erythroderma – 0.67 ng/ml
4. Erythema multiforme major – 0.125 ng/ml (IQR 0.085 – 0.18)
5. Generalised FDE – 0.1 ng/ml

6. Controls – 0.27 ng/ml (IQR 0.1325- 0.5225)

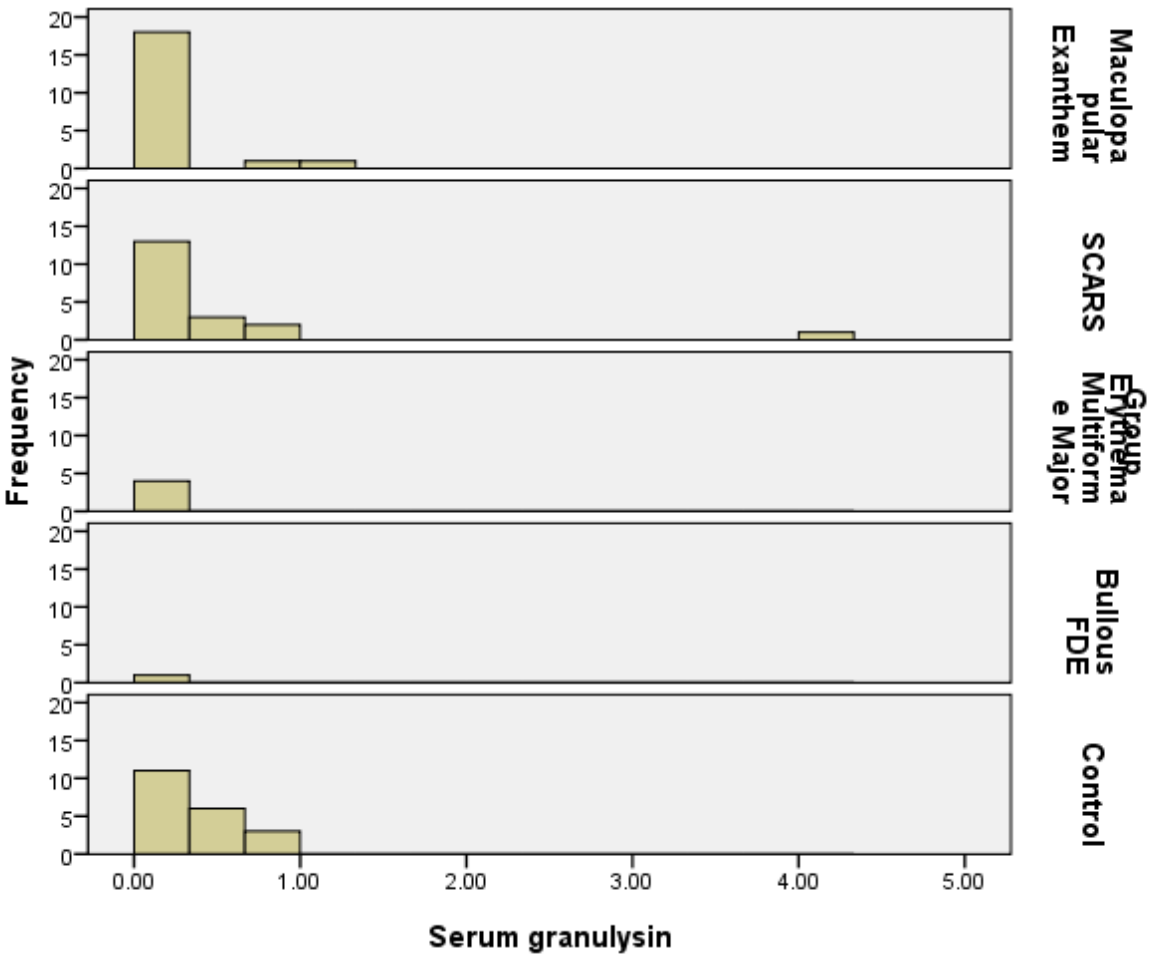


Figure 19 - Distribution of serum granulysin in different CADRs

There was no statistically significant difference in the serum granulysin titer between the MPE group and SCARs group or between the SCARs group and controls.

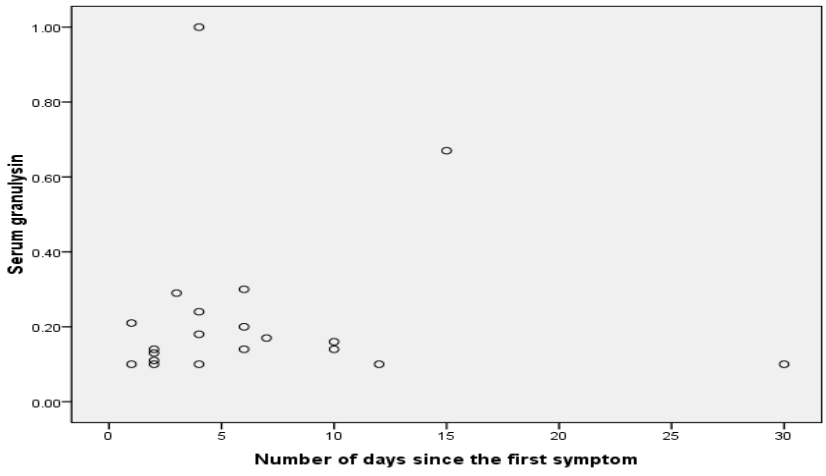
An interesting finding in the study was that there was a significant difference in the serum granulysin between SJS/TEN and erythema multiforme major patients (p value – 0.042).

7.2 Steroid administration in patients with CADRs and serum granulysin levels :

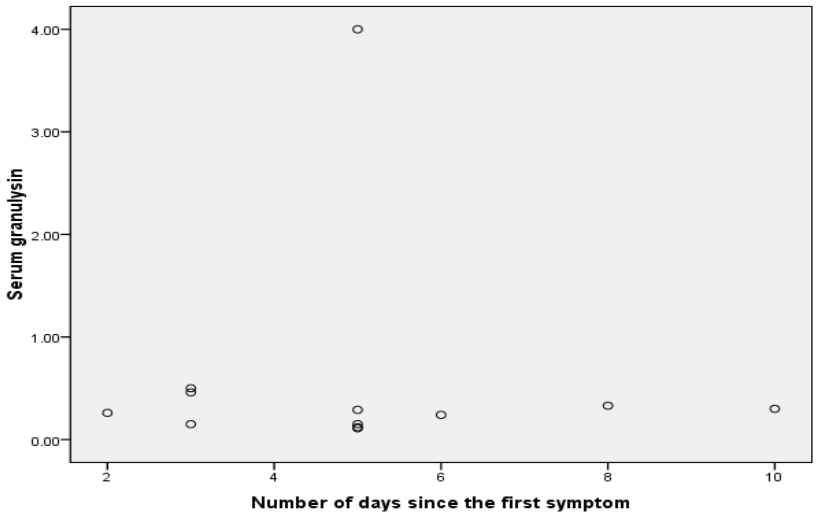
The serum granulysin levels were not influenced by the administration of systemic steroids in any of the groups. In fact, in the SCARs group, the patients who were on systemic steroids at the time of inclusion in the study had a higher median value of serum granulysin than the patients who were not on steroids (0.295 *vs* 0.24).

7.3 Relationship between serum granulysin level and the time of presentation

The serum granulysin titer did not appear to be dependent on the number of days since the onset of the rash in MPE, SJS/TEN or DRESS. The pattern of distribution of serum granulysin did not show significant elevation in patients who presented during the early phase of illness as compared to patients who presented in the late stages **(figure 20)**.



MPE



SJS/TEN

Figure 20 – Serum granulysin vs day of initial presentation

CLINICAL PHOTOGRAPHS

a – mucosal erosions in a patient with erythema multiforme major

Target lesions on the forearm (b) and palm (c) of a patient with erythema multiforme major

d – epidermal detachment in a patient with SJS-TEN overlap

e – erythematous papules and atypical target lesions on the distal aspect of arm of a patient with SJS

f – diffuse cheilitis and atypical target lesions in a patient with SJS

g – conjunctival suffusion in SJS

h – facial and periorbital oedema in DRESS syndrome

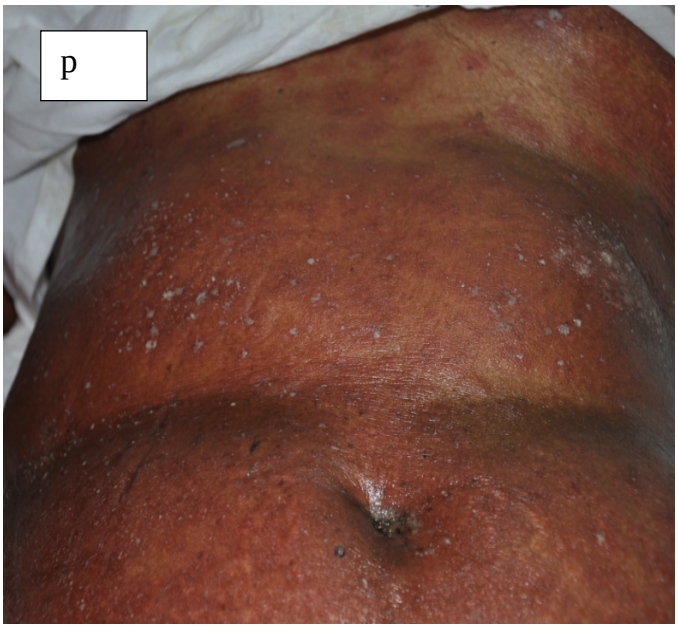
i – infiltrated papules and papulovesicles on the forearm of a patient with DRESS

j – fine scaling and erythema in a patient with drug induced erythroderma

k – cheilitis and facial oedema in a patient with maculopapular exanthema

Photo-distributed rash secondary to chlorpromazine with a sharp demarcation at the covered sites (l,m)

Dull erythematous plaques (n) and overlying bulla (o) in a patient with generalized fixed drug eruption



Erythematous oedematous plaques studded with pinpoint pustules on the abdomen (p). Pustules in the same patient resolving with characteristic desquamation (q)

DISCUSSION

Serum granulysin is a molecule that has been under extensive study for its antibacterial and immunologic properties. The focus on serum granulysin as a marker for cutaneous adverse drug reactions has been recent, and there are few studies which have compared the levels of granulysin in the different types of CADRs (47,63). To the best of our knowledge, there are no Indian studies that have compared the serum granulysin levels in patients with drug induced MPE, SCARs, drug induced EMM and healthy controls. Our study aimed to explore the relationship between serum granulysin and the severity of CADRs and to study the clinical profile of patients with cutaneous adverse reactions.

1. DEMOGRAPHIC PROFILE :

A comparison of the demographic profile of the patients in this study with other Indian studies is tabulated below **(table 13).** It is noteworthy that the incidence of AGEP was low in all these studies, similar to our study. This could be because AGEP generally resolves spontaneously in a short period after discontinuation of the drug, rarely necessitating treatment. The relative paucity of SCARs in our patient group could be because of the exclusion of patients with CADRs to anti-tuberculous and anti-retroviral therapy.

Table 13 - Comparison of demographic profile of patients with CADRs in different Indian studies

Parameter	Pudukadan et al (31)	Sasidharanpillai et al (34)	Sharma VK et al (35)	Present study
Country	India	India	India	India
Study period	2001-2003	2011-2012	1991-1996	2015-2017
Study duration	2 years	1 year	6 years	2 years
Study design	Prospective study	Prospective study	Prospective study	Prospective case-control study
Population	South India	South India	North India	India, Bhutan, Bangladesh
Age (mean ± SD) years	37.06 ± 30.12	NA	34.5	44.4 ± 14.6
M : F	0.87 : 1	1.5 : 1	1.47 : 1	0.41 : 1
CADR(n)	SJS/TEN - 17	SJS/TEN - 17	SJS/TEN - 57	SJS/TEN - 12
	DRESS - 0	DRESS - 7	DRESS - 0	DRESS - 7
	AGEP - 2	AGEP - 1	AGEP - 0	AGEP - 1
	Erythro - 3	Erythro - 2	Erythro - 9	Erythro - 1
	MPE - 11	MPE - 8	MPE - 173	MPE - 22
	EMM - 6	EMM - 3	EMM - 22	EMM - 4
	FDE - 28	FDE - 4	FDE - 150	FDE - 1

Erythro – drug induced erythroderma

In these studies, other forms of CADRs such as urticaria and angioedema, lichenoid drug eruption and photosensitive reactions were also included. The type of CADRs included in each study is enclosed **(Annexures 8).**

1.1 **Age :** The mean age of the patients recruited in our study was 44.4 ± 14.6 years, which was similar to other studies on cutaneous adverse drug reactions (91,92). In this cohort, 46% of the patients belonged to the age group of 40-60 years, which was in concordance with data published in previous studies (92–94). This trend could be due to the higher incidence of lifestyle associated diseases and subsequent polypharmacy in this age group, the increased risk of drug interactions and the altered metabolism and elimination of drugs from the body. However, there are Indian studies which have reported cutaneous drug reactions to be more common below 40 years of age (7,91). This has been attributed to the increased consumption of antibiotics in the younger age group. In our study group, 40% of patients were between the ages of 18 and 40 years **(fig 5).**

1.2 **Gender** - There was a significant female preponderance in our study with a male : female ratio of 0.41:1 **(fig 6).** Many previous studies have shown a higher female : male ratio among patients with cutaneous adverse reactions (4,91,93). We also analysed the gender breakdown among the main groups of CADRs – MPE, SCARs, EMM and FDE – and found that the majority of the patients with MPE and SCARs were female **(fig 7).** This is contrary to the data published by Sasidharanpillai et al, which showed a male predominance in all SCARs except DRESS (94). The gender disparity in our study could reflect a complex interplay of socio-economic and cultural issues which affects the health-seeking behaviour in our population. For instance, women may consume more medications over the counter than men, resulting in higher incidence of drug

reactions (4). Also, men, being the primary wage earners in the family, may not seek treatment for non-severe drug reactions. The perception of illness may also be different between the genders.

1.3 Day of presentation - More than half (58%) of the patients presented early in the course of the disease, i.e, within 5 days of onset of symptoms **(fig 11).** Only 15% patients (n=7) presented after more than 10 days of onset of symptoms. Three out of these seven patients (42.8%) had sought treatment elsewhere prior to this presentation. Among the male patients, approximately 94% sought medical attention within 10 days of onset of complaints whereas among the female patients, this percentage was lower (81%). This implies that a larger proportion of female patients sought medical attention later than their male counterparts.

1.4 **Latent period** – The mean latent period for the DRESS patients in this study was markedly longer (16.1 ± 10.3 days) than MPE (8.4 ± 7.2 days). However, this difference was not statistically significant (p value 0.1032). Nevertheless, this is consistent with the observation that DRESS manifests 3-8 weeks after the ingestion of the offending drug. It is also noteworthy that despite the low sample size, the AGEP and FDE patients reported a mean latent period of 1 day, which conforms with data from literature (15) **(table 5).**

2. DRUG DETAILS :

The Naranjo Adverse Drug Reaction Probability Scale **(Annexure 5)** was used to assess causality of CADRs. Antiepileptic drugs were the commonest group of offending drugs (35.4%) among our patient population **(fig 12).** This was similar to the results of previous reports (5,44,51). Carbamazepine was

the most frequently encountered drug (20.83%, n=10), closely followed by phenytoin (18.75%, n=9). Levetiracetam, phenobarbitone and valproate were also reported to a lesser extent. Approximately 19% patients reported various antibiotics as the suspected drug. The most common antibiotics were beta lactams (penicillins, carbapenems and cephalosporins). This data conforms with findings from past studies (95,96).

An interesting observation which emerged in this study was that antiepileptic agents were implicated as causative agents in 47.6% SCARs whereas antibiotics were the common culprit drug group (27.3%) among MPE. A study from Eastern India has made similar observations with antiepileptic drugs. In this study, 59% CADRs secondary to antiepileptic agents were SCARs (97).

The other drugs identified in our study were proton pump inhibitors such as pantoprazole, diuretics such as hydralazine, torsemide and sulfa drugs (sulfasalazine, dapsone). Traditional or alternative medicine may also contribute to CADRs in our country (98). Among our study population, there was a maculopapular exanthema which was suspected to be secondary to siddha treatment.

Literature shows anti-retroviral drugs and anti-tuberculous drugs as common causes of CADRs (99–101). But these groups of drugs were under-reported in our cohort as patients with confirmed diagnosis of tuberculosis and HIV were excluded from our study due to the implicated role of serum granulysin in these chronic infections (102–104).

One of our patients had maculopapular skin lesions strictly confined to the exposed areas of the face and upper limbs **(*clinical photograph – l,m*).** The suspected culprit drug was chlorpromazine, which is

a well recognised phototoxic and photoallergic drug (105,106). Chlorpromazine is known to cause severe persistent photosensitivity even after cessation of exposure to the drug (107).

A maculopapular rash secondary to rabies vaccine/anti-rabies immunoglobulin was seen in one of our patients. Another patient who had been treated with carbamazepine and rituximab for neuromyelitis optica spectrum developed SJS. (108). This patient was also on carbamazepine for three weeks preceding the onset of symptoms and it may have been the culprit drug. However, the possibility of a Rituximab induced SJS could not be excluded (Naranjo causality score - 2). Rituximab induced Stevens-Johnson syndrome was first reported in 2002 by Lowndes et al (109). This diagnosis was later challenged by Henning and Firoz (108).

Five patients in the cohort had a past history of drug allergy. Pichler et al has reported a higher incidence of DRESS in patients with a past history of drug reactions(110), but this was not seen among our patient population.

3. CLINICAL FEATURES :

Cutaneous adverse drug reactions can have varied manifestations. The most common symptoms at presentation in our study were fever with mucocutaneous involvement **(fig 9).** Previous studies have established facial oedema as a hallmark in patients with DRESS syndrome and other SCARs (1,94,111). Among the DRESS patients in our study, the frequency of facial oedema was slightly higher (33.3%) than what has been previously described by Husain et al, i.e, 25% (112). However, this difference was not statistically significant. In our study, facial oedema was present in 28.57% of

SCARs and 22.7% of MPE (p value - 0.929) **(table 6).** Thus, facial oedema, which has heretofore been described as a classical feature of DRESS syndrome and other SCARs, may also be seen in non-severe CADRs. Blanching erythema, purpuric or atypical target lesions, erosions, erythematous papules and desquamation were the other facial features seen in our patients.

4. SYSTEMIC MANIFESTATIONS :

The most common systemic manifestation in our population was haematological abnormalities like lymphocytopenia followed by peripheral eosinophilia. Systemic features were more common among patients with SCARs. The laboratory parameters of our patients are compared with patients from other Indian studies in **table 14**.

Table 14 - Comparison of laboratory parameters of patients with CADRs in different studies

Parameter	Pudukadan et al (31)	Sasidharanpillai et al (34)	Sharma VK et al (35)	Present study
Peripheral eosinophilia	42.2% (n=38)	DRESS – 100%	NA	SCARs – 35% MPE – 36.4%
		SJS/TEN – 29.4%		
		Erythroderma – 50 %		
		MPE, EMM, AGEP - 0 %		
Abnormal LFT	Severe CADR – 88.9% Non severe CADR – 11.11%	DRESS – 100%	NA	MPE – 4.5% SCARs – 33.3%
		SJS/TEN – 35.2%		
		MPE – 12.5%		

		EMM – 33% Erythroderma, AGEP - 0 %		
Most common drugs	Cotrimoxazole Dapsone	Phenytoin Carbamazepine	Cotrimoxazole Phenytoin	Carbamazepine Phenytoin

4.1 **Lymphadenopathy** - Lymphadenopathy was seen among 13.6% patients with MPE and 19% patients with SCARs (p value - 0.698). Among the patients with DRESS, 33.3% had enlarged nodes, a figure much lower than that reported in previous studies (44,52).

4.2 **Hepatic dysfunction** – Transaminitis was present in 33.3% of patients with SCARs as compared to 4.5% MPE. Thus, hepatic involvement was significantly more common in SCARs than MPE (p value – 0.021).

4.3 **Pulmonary involvement** - Cough, dyspnea and chest radiograph changes suggestive of pulmonary involvement were noted in 9 out of the 48 patients (18.75%). Of these, 5 patients (55.5%) were SJS/TEN patients. One patient had erythema multiforme major. The other 3 patients had MPE. The most common lung findings noted were pulmonary infiltrates (55.5%, n=5) suggestive of interstitial pneumonitis. The incidence of pulmonary involvement in SCARs was higher than in MPE, but not statistically significant (p value – 0.098). Bronchiolitis obliterans, eosinophilic pneumonia, vasculitis and alveolar injury are the other drug induced pulmonary changes that have been reported in the past (113,114). However, none of these changes were observed in this cohort. It is also to be noted that 41.6% (n=5/12) of SJS/TEN patients had some form of pulmonary involvement.

4.4 **Renal involvement** – Severe cutaneous adverse reactions are associated with renal changes such as glomerulonephritis, late hypokalemia (36), interstitial nephritis (12), tubular necrosis (34) and acute renal failure (115). In our study, raised creatinine was seen in 6 patients, with equal number of patients in the MPE and SCARs group (p value – 0.345) **(table 11**). We noted that 25% of our SJS/TEN patients had abnormal renal parameters, similar to the data published in previous studies (36). Urine eosinophils was present only in 3 patients, all of whom had MPE. However, the specificity of urine eosinophil count for detection of interstitial nephritis is low (116).

4.5 **Cardiovascular involvement** – Features suggestive of carditis were seen in one patient with DRESS (n=1/7, 14.3%). Kardaun et al reported a 13% incidence of complications in the heart or muscles in their landmark study on DRESS patients (44). Ampicillin, aromatic anticonvulsants and allopurinol have been most commonly associated with carditis in DRESS (117). In our patient, phenobarbitone was the suspected drug.

4.6 **Haematological abnormalities** – Haematological abnormalities were fairly common among patients with all types of CADRs **(table 11).**

a) **Lymphocytopenia** - The most common changes seen were lymphocytopenia, which was present in 68% of the total number of patients, 59% MPE and 80% SCARs (p value – 0.232). Lymphocytosis was not seen in any of our patients. Kardaun et al reported lymphocytosis and lymphopenia among 52% and 5% DRESS patients respectively.

b) **Thrombocytopenia**– this finding was far more common among SCARs than MPE (28.6% vs 9%). However, this difference was not statistically significant (p value – 0.132).

c) **Eosinophilia**– Peripheral eosinophilia has traditionally been considered as an important diagnostic clue for drug induced aetiology. In this cohort, 36.4% patients with MPE and 35% of patients with SCARs had eosinophilia (p value > 0.99) **(fig 18)**, which was lower than the figures noted in previous studies (31,34,39). It is also to be noted that contrary to popular belief, only a third of the patients with CADRs might have peripheral eosinophilia. Hence, the absence of peripheral eosinophilia may not rule out a drug induced etiology (8). This data also confirms the hypothesis that peripheral eosinophilia is associated with diffuse cutaneous reactions like MPE and SCARs as previously reported (118), regardless of the specific morphology or systemic involvement.

Two important subsets of our patient population were SJS/TEN and DRESS cases. A comparison of the clinical profile of SJS/TEN patients in our study and few other studies in literature are enumerated below in **table 15**.

Table 15 – Comparison of clinical parameters of SJS/TEN patients in different studies

Parameter	Wang L et al (119)	Chantaphakul et al (120)	Wong et al (121)	Sharma et al (122)	Naveen et al (123)	Present study
Country	China	Thailand	Australia	India	India	India
Study period	2006 - 2015	Published in 2015	1985 - 1997	2003 - 2006	Published in 2013	2015 - 2017
Study duration	10 years	5 years	12 years	4 years	5 years	2 years
Study design	Retrospective	Retrospective	Retrospective	Retrospective	Retrospective	Prospective
Subjects (n)	88	43	17	30	22	12
M : F	0.83 : 1	1.4 : 1	1.83 : 1	0.76 : 1	1.75 : 1	0.16 : 1
Age (mean ± SD) years	45 ± 18	49.5 (range 20-85)	61.5 (range 23–83)	22.3 ± 15.4	32.3 (range 1- 65)	44.4 ± 14.6
Common drugs	Antibiotics	Allopurinol	Antibiotics	AED	AED	AED
Visceral involvement	47.7%	48.8%	NA	NA	NA	66.67%

AED – antiepileptic drugs

All the studies mentioned in the comparison were retrospective studies. The mean age of our study patients (44.4 ± 14.6 years) was similar to that of the other studies, except 2 studies which included

pediatric patients. The most commonly implicated drugs in all the Indian studies were antiepileptic agents. Systemic involvement was higher (66.67%) among the patients included in our study.

A similar comparison of DRESS patients in our study with other studies is shown in **table 16**.

Table 16 - Comparison of demographic and clinical profile of patients with DRESS in different studies

Parameter	Kardaun et al (44)	Lee JY et al (124)	Eshki et al (45)	Present study
Country	Europe	Korea	France	India
Study period	2003 - 2009	2006 - 2015	1995 - 2006	2015 - 2017
Study duration	7 years	10 years	12 years	2 years
Study design	Prospective study	Retrospective study	Retrospective study	Prospective case control study
Subjects	DRESS (n = 117)	DRESS (n=25)	Severe DRESS (n = 15)	DRESS (n=7)
M : F	0.80 : 1	0.78 : 1	NA	0.75 : 1
Drugs implicated	Carbamazepine, allopurinol	Carbamazepine, allopurinol	Allopurinol, minocycline	carbamazepine
Facial oedema	76%	28%	NA	28.6%
Mucosal lesions	56%	20%	NA	42.9%
Lymph node enlargement	54%	64%	NA	42.9%
Abnormal LFT	75%	80%	46.7%	57.1%
Abnormal RFT	37%	28%	33.3%	0%
Pulmonary involvement	32%	20%	66.7%	0%

Peripheral eosinophilia	95%	80%	NA	71.4%%
Lymphocytopenia	5%	NA	NA	14.3%
Lymphocytosis	52%	NA	NA	0%
Thrombocytopenia	7%	40%	NA	28.6%
Thrombocytosis	19%	NA	NA	0%

The total number of DRESS patients in our study was lesser than the other studies in this comparison. There was a female preponderance in our study group similar to other studies. The most commonly implicated drugs were aromatic anticonvulsants like carbamazepine. Mucosal involvement was seen in 42.9% of our patients. The incidence of facial oedema was lesser in this study as compared to the data published by Kardaun et al. In contrast, lymphocytopenia and thrombocytopenia were more commonly seen.

5. HISTOPATHOLOGY :

Few characteristic histopathological features were seen in drug induced reactions.

5.1 Dermal eosinophilia - Of the 24 patients who underwent histopathological analysis, dermal eosinophilic infiltrate was the most commonly encountered finding. In the study by Weinborn et al, the prevalence of dermal eosinophilia was 61% as opposed to 83.3% in our study (63). This confirms that peripheral eosinophilia does not necessarily correlate with dermal eosinophilia (118) as already reported in previous studies. The incidence of dermal eosinophilia was comparable among the MPE and SCARs groups in this study (p value > 0.99). Two out of the 3 DRESS patients (66.67%) who

underwent histopathological analysis showed dermal eosinophilic infiltrate, similar to the data published earlier (125). In this study, Weinborn et al also reported basal cell vacoulization in 50% cases of DRESS.

5.2 Necrotic keratinocytes - In our study, necrotic keratinocytes were seen in 100% patients with SCARs whereas lymphocytic exocytosis was seen in 90% patients with SCARs. This is in conformity with the observations of Weinborn et al (125). Both necrotic keratinocytes and lymphocytic exocytosis were significantly more common in SCARs than MPE (p value – 0.01 and 0.045 respectively).

Thus, interface vacoulization, lymphocytic exocytosis, dyskeratosis and spongiosis were all more common among the SCARs than in MPE as shown previously by Chi et al (65).

The findings in our study also confirms that there are no characteristic histopathological features in MPE(63). The usual findings noted are spongiosis, dermal oedema and a perivascular infiltrate.

6. ROLE OF SERUM GRANULYSIN :

Serum granulysin levels of different CADRs were measured and compared. The median granulysin concentration in patients with SJS/TEN in our study was lower than that reported by Fujita et al (86). Among the DRESS patients in our study, the median granulysin level was 0.24 ng/ml, which was lower than that detected by Saito et al. The test kit used was different, hence, a direct comparison cannot be done.

The study characteristics of our study are compared with existing literature in **table 17.**

Table 17 - Comparison of study characteristics of different studies on serum granulysin in CADRs

	Present study	Fujita et al (86)	Saito et al (47)
Country	India	Japan	Japan
Study period	November 2015 – May 2017	NA (published in 2011)	NA (published in 2012)
Study duration	19 months	NA	NA
Study design	Single center, case control study	Multi center, case-control study	Multi center, case-control study
Number of patients (cases)	n = 21 SCARs + 22 MPE + 4 EMM+ 1 FDE	n = 5 SJS/TEN	n = 15 DRESS
Day of inclusion	At first presentation (ranged between day 1 to day 30)	2-4 days prior to epidermal detachment	Serial monitoring done – day 1-10, day 11-20, day 21 and above
Controls	20 healthy controls	24 patients with ODSRs (specific type NA), 31 healthy controls	24 patients with MPE/ EMM, 31 healthy controls
Type of test	ELISA	Rapid immunochromatographic strip test and ELISA	ELISA
Range of detection of serum granulysin	0.03 ng/ml to 4 ng/ml	NA (granulysin titer ranged between 2.7 ng/ml – 52.1 ng/ml)	NA (granulysin titer ranged between 1.6 ± 0.6 ng/ml to 21.9 ± 12 ng/ml
Test kit used	Biovendor	Recombinant granulysin,	MBL, Nagoya,

	RD191327200R Human Granulysin, Czech Republic	R&D Systems, Minneapolis, MN	Japan

Existing literature shows that granulysin is highly expressed in the blister fluid seen in SJS/TEN (30). Further studies reported serum granulysin to be elevated in the early stages of SJS/TEN (with rapidly falling titers within few days of epidermal detachment) as compared to ODSRs. But this finding was not reproduced in our study. Among the 12 patients with SJS/TEN, serum granulysin > 4 ng/ml (upper limit of detection) was only seen in one patient, which remained persistently high on day 3. Most of our patients had epidermal detachment at initial presentation and hence, serial measurement of granulysin was not performed for all patients with SJS/TEN.

In contrast, studies have shown a prolonged elevation of serum granulysin in DRESS (47). This was also not seen among our patients. In our study, the granulysin titers were comparable among the MPE, SCARs, EMM, drug induced erythroderma and healthy controls. We cannot comment on serum granulysin in bullous FDE and AGEP due to the low sample size.

6.1 Comparison of serum granulysin in SJS/TEN and EMM

An interesting observation that emerged in our study was the difference in granulysin levels of SJS/TEN and EMM. It has also been established that there is a significant difference in the expression of granulysin in the tissue of patients with SJS/TEN and EMM (71). At the onset of the disease, both

erythema multiforme major and Stevens Johnson syndrome present with purpuric or target lesions, which may further progress to mucocutaneous erosions. However, EMM is self-limiting and symptomatically less severe as compared to SJS/TEN. In our study, we found that serum granulysin was significantly higher in SJS/TEN than EMM (p value – 0.042), and can therefore be used to distinguish between the two conditions.

At the end of our study, we concluded that serum granulysin may not be uniformly raised in SCARs like SJS/TEN and DRESS as reported earlier. Further studies with larger sample size need to be done to reassess the role of granulysin in various CADRs.

CONCLUSION

1. In this study, there were forty eight patients with various cutaneous adverse reactions including maculopapular exanthema (n = 22), erythema multiforme major (n = 4), fixed drug eruption (n = 1), SJS/TEN (n = 12), DRESS (n = 7), drug induced erythroderma (n = 1) and AGEP (n = 1).

2. The mean age was 44.4 ± 14.6 years with the most common age group affected being 40-60 years (n=22/48, 46%).

3. There was a strong female preponderance (M:F 0.41:1) with 68.18% MPE patients (n=15/22) and 76.19% SCARs patients (n=16/21) being women.

4. The most common drug group implicated among all the CADRs in our study was antiepileptic drugs (n=17/48, 35.4%). Carbamazepine was the most commonly reported drug (n=10/48, 20.83%).

5. In our study, antiepileptic drugs were the most commonly implicated drugs in SCARs (n=10/21, 47.6%) and antibiotics were the most commonly implicated drugs in MPE (n=6/22, 27.3%).

6. The prevalence of facial oedema among patients with MPE and SCARs was comparable (n=5/22, 22.7% *vs* n=6/21, 28.57%, p – 0.929).

7. Palmoplantar involvement was more common in patients with SCARs (n=9/21, 42.85%) than in patients with MPE (n=3/22, 13.6%).

8. There was no significant difference in the frequency of peripheral eosinophilia among MPE and SCARs (36.4% *vs* 35%, p > 0.99).

9. Peripheral eosinophilia was found only in about one third of the patients with CADRs (n=15/48, 31.25%) and thus, the absence of peripheral eosinophilia may not rule out drug induced aetiology.

10. Transaminitis was significantly more common in SCARs than MPE with (n=7/21, 33.3% vs n=1/22, 4.5%, p value 0.021).

11. Lymphocytopenia (80% SCARs and 59.1% MPE) and thrombocytopenia (28.6% SCARs and 9.1% MPE) were seen more in patients with SCARs in comparison to those with MPE. However, this was not statistically significant (p value 0.232, 0.132 respectively).

12. The most frequently encountered histopathological feature in CADRs was dermal eosinophils (n=20/24, 83.3%).

13. The occurrence of spongiosis (n=7/10, 70% SCARs and n=7/12, 58.3% MPE) and dermal eosinophilia (n=9/10, 90% SCARs and n=10/12, 83.3% MPE) among SCARs and MPE were comparable (p – 0.321, > 0.99 respectively).

14. Necrotic keratinocytes on histopathology was seen in all the cases of SCARs (n=10/10, 100%).

15. Necrotic keratinocytes were significantly more common in the histopathology of SCARs (n=10/10, 100%) as compared to MPE (n=5/12, 41.6%) (p – 0.01).

16. Lymphocytic exocytosis was more commonly seen in SCARs than MPE (n=9/10, 90% vs n=6/12, 50%, p – 0.045).

17. There was no significant difference in serum granulysin between patients with maculopapular exanthema (median – 0.15 ng/ml), severe cutaneous adverse reactions (median – 0.26 ng/ml) or healthy controls (0.27 ng/ml). Thus, serum granulysin may not be specific for SJS/TEN as thought previously.

18. There was no correlation between serum granulysin and the severity of the cutaneous adverse drug reaction.

19. Granulysin titer was significantly higher in patients with SJS/TEN than in patients with erythema multiforme major (0.275 ng/ml *vs* 0.125 ng/ml, p value – 0.042).

LIMITATIONS

1. In patients with established epidermal detachment, serial measurement of granulysin was not done.
2. The range of detection of serum granulysin was lower in our test kit than that used in other studies.

RECOMMENDATION

Multi-center studies with larger number of patients should be conducted to assess the levels of granulysin in CADRs and its correlation with the severity of the rash.

Serum granulysin should be correlated with the granulysin expression in skin in patients to ascertain the pathogenetic role of granulysin in cutaneous adverse drug reactions.

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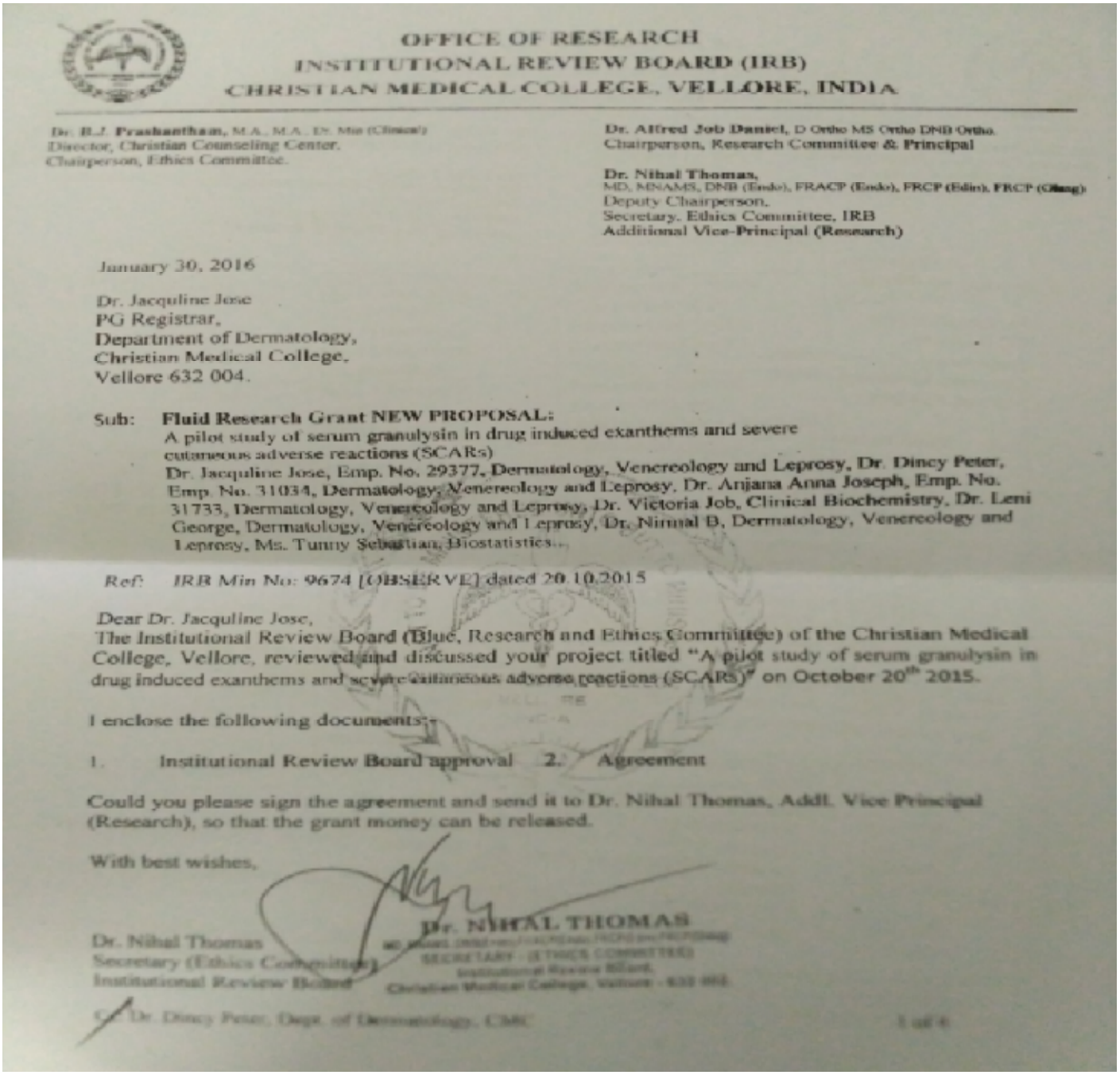
ANNEXURE 1 – IRB APPROVAL



OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center.
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD, MNAMS, DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

January 30, 2016

Dr. Jacquline Jose
PG Registrar,
Department of Dermatology,
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)
Dr. Jacquline Jose, Emp. No. 29377, Dermatology, Venereology and Leprosy, Dr. Dincy Peter, Emp. No. 31034, Dermatology, Venereology and Leprosy, Dr. Anjana Anna Joseph, Emp. No. 31733, Dermatology, Venereology and Leprosy, Dr. Victoria Job, Clinical Biochemistry, Dr. Leni George, Dermatology, Venereology and Leprosy, Dr. Nirmal B, Dermatology, Venereology and Leprosy, Ms. Tunny Sebastian, Biostatistics.

Ref: *IRB Min No: 9674 [OBSERVE] dated 20.10.2015*

Dear Dr. Jacquline Jose,
The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)" on October 20th 2015.

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. NIHAL THOMAS

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Cc: Dr. Dincy Peter, Dept. of Dermatology, CMC

OFFICE OF RESEARCH
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The Committee reviewed the following documents:

1. IRB Application format
2. Data Collection proforma
3. Naranjo Adverse Drug Reaction Probability Scale
4. Patient Information Sheet and Informed Consent Form (English, Tamil, Hindi)
5. Cvs of Dr Dincy Peter, Dr. Victoria Job Ms. Tunny Sebastian
6. No. of documents 1 – 5

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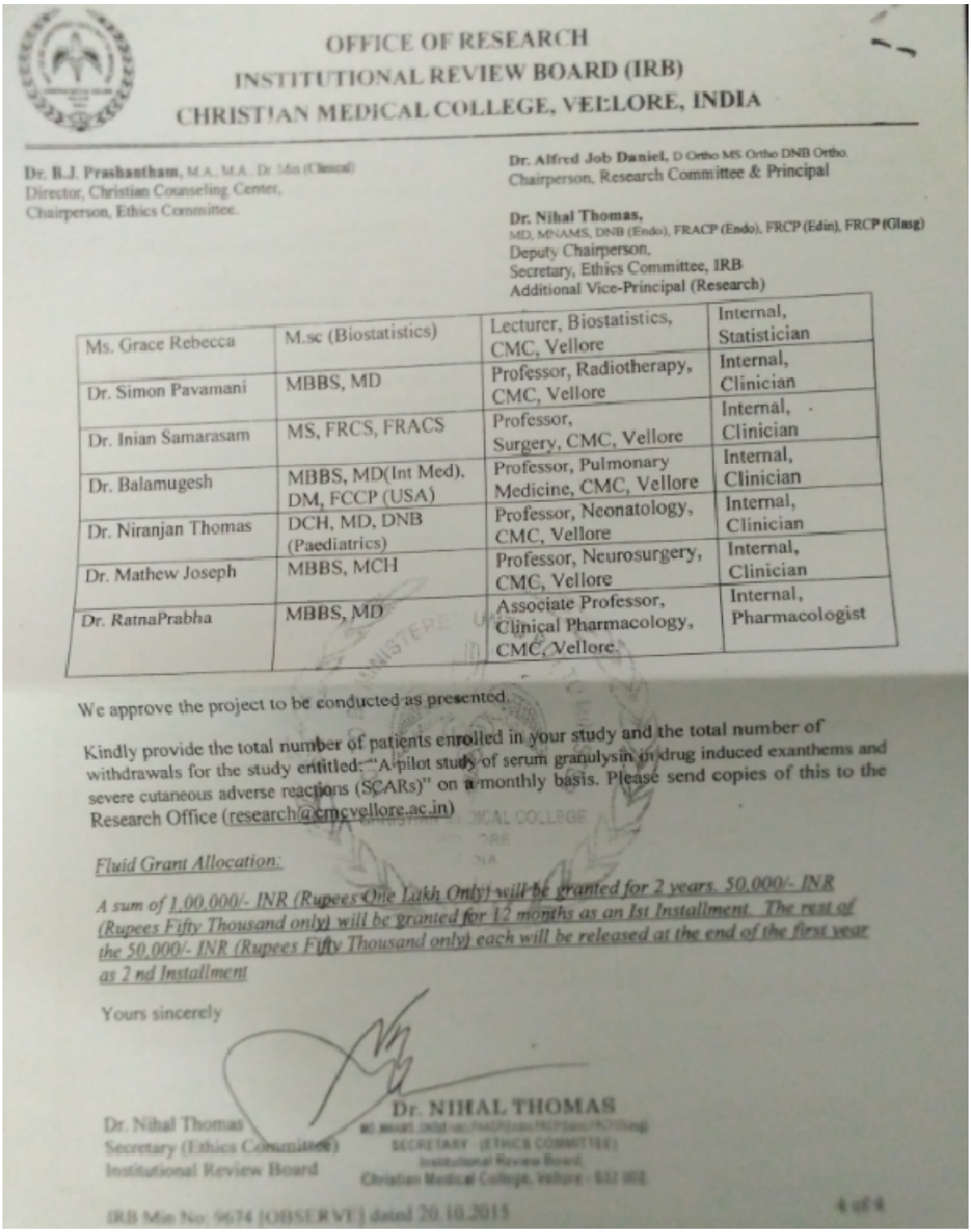

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Dr. Niranjan Thomas	DCH, MD, DNB (Paediatrics)	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician
Dr. RatnaPrabha	MBBS, MD	Associate Professor, Clinical Pharmacology, CMC, Vellore.	Internal, Pharmacologist

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in)

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment

Yours sincerely

Dr. NIHAL THOMAS
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board
Christian Medical College, Vellore - 632 002

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

IRB Min No: 9674 [OBSERVE] dated 20.10.2015

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ANNEXURE 2 - INFORMATION SHEET

Study Title: A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)

CHRISTIAN MEDICAL COLLEGE, VELLORE - DEPARTMENT OF DERMATOLOGY

Explanation of the purpose of research?

Drug eruptions are very common and usually occur within a few weeks of starting new medications. While majority of these are harmless and resolve in a few weeks with supportive management, a small proportion can evolve into life threatening disease. In the initial stages, all drug eruptions appear similar. Identifying the patients who are at risk of progressing into a severe disease early would help the doctor to manage the patient aggressively with systemic and topical treatment in an in-patient set up, thus potentially reducing the death rate. Granulysin is a molecule which was identified as a key mediator responsible for the skin manifestations in severe drug eruptions. It is increased in the blood in patients before the appearance of the rash. This study will help us to show the relationship between granulysin and the different types of drug eruptions. This may help us to recommend granulysin as a predictor of severe drug eruptions in patients who present early in the course of the illness.

What will you have to do if you participate in his study?

If you agree to participate in this study once you have been diagnosed to have drug eruptions, you will be requested to allow a doctor to take a detailed history and examination. You are also required to give blood sample for the measurement of serum granulysin. In case of worsening lesions, you will be requested to give a second blood sample. The detected serum granulysin level is then correlated with disease severity and compared with that of healthy individuals.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in anyway.

What will happen if you develop any study related injury?

We do not expect any injury to happen to you but if you do develop any side effects or problems due to the study these will be treated at no cost to you.

Will you have to pay for the blood test?

The test serum granulysin concentration will be done for you free of cost.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of results. However your medical notes may be reviewed by people associated with the study without your additional permission should you decide to participate in this study.

ANNEXURE 3 - INFORMED CONSENT FORM

Study Title: A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)

Study Number: _____

Subject's Name: _____

Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

Signature (or Thumb impression) of the Subject/Legally Acceptable

Date: _____/_____/_____

Signatory's Name: _____ Signature:

Or

Representative: _____

Date: _____/_____/_____

Signatory's Name: _____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

ANNEXURE 4 - CLINICAL RESEARCH FORM

A pilot study of serum granulysin in drug induced exanthems and severe cutaneous adverse reactions (SCARs)

Date: Serial no:

Name: **Hospital no:**

Age:

Sex: Male / Female Occupation:

Address:

State:

Contact number:

Presenting complaints:

Day since the onset of the first skin lesion -

1. Duration of symptoms -

Symptom	Yes	No	Duration
Itching			
Burning sensation			
Conjunctival lesions			
Oral mucosal lesions			
Genital mucosal lesions			
Others			

2. **Systemic complaints:**

Symptoms	Yes	No	Duration
Fever			
Cough			

Breathlessness			
Jaundice			
Arthralgia			
Swelling of face /hands and feet			

Drug/drugs implicated-

Indication for the initiation of these new drug/drugs -

Type of the implicated drug – anticonvulsants / antibiotics / analgesics / antiretroviral / antituberculous drugs

Sl.no	Medication	Initiation date	Date of stoppage	Duration	Half life

Concurrent drugs:

Serial number	Drug	Duration	Other comments

Previous history of drug reaction: yes/no

If yes, then details:

Serial no	Drug name	Type of reaction	Date of previous reaction

Drug timeline -

Drug	-5	-4	-3	-2	-1	Day 0	+1	+2	+3
1.									
2.									
3.									
4.									

Is the patient on systemic steroids? Yes / No

If yes, duration :

Indication :

Other co-morbidities-

Personal history:

Alcohol, if yes: duration_____ (in years) and amount_____ (in ml)

Clinical Features:

Fever:

Heart rate:

Respiratory rate:

Blood pressure:

Lymphadenopathy:

Respiratory system:

Gastrointestinal system:

Type of lesion: Blanching erythema / macular / papular / maculopapular / purpuric / erythema multiforme-like (targetoid) / vesicles / pustules / bullae / erosions

Body surface area:

<10%	
10% -30%	
>30%	

Scaling : Yes / No

Palms and soles : yes / no

If yes, describe :

Face involvement : yes / no

If yes, describe :

Mucosal involvement : yes / no

If yes,describe :

Eyes :

Oral cavity :

Genitalia :

Nail changes : yes / no

If yes, describe :

Hair / scalp changes : yes / no If yes,

describe :

Others :

Investigations

	Day no. _
Haemoglobin	
Platelets	
Total counts	
Differential counts	
Total bilirubin	
Total protein	
Serum albumin	
SGOT	
SGPT	
Alkaline phosphatase	
Serum creatinine	
Urine for eosinophils	
Chest X ray findings	
HIV	
Serum granulysin	

Repeat granulysin - Yes/No

If yes, value :

OTHERS:

Histopathology findings with date :

ANNEXURE 5 - NARANJO ADVERSE DRUG REACTION PROBABILITY SCALE

Naranjo Adverse Drug Reaction Probability Scale				
Question	Yes	No	Do Not Know	Score
1. Are there previous *conclusive* reports on this reaction?	+1	0	0	
2. Did the adverse event appear after the suspected drug was administered?	+2	-1	0	
3. Did the adverse reaction improve when the drug was discontinued or a *specific* antagonist was administered?	+1	0	0	
4. Did the adverse event reappear when the drug was re-administered?	+2	-1	0	
5. Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	+2	0	
6. Did the reaction reappear when a placebo was given?	-1	+1	0	
7. Was the drug detected in blood (or other fluids) in concentrations known to be toxic?	+1	0	0	
8. Was the reaction more severe when the dose was increased or less severe when the dose was decreased?	+1	0	0	
9. Did the patient have a similar reaction to the same or similar drugs in *any* previous exposure?	+1	0	0	
10. Was the adverse event confirmed by any objective evidence?	+1	0	0	
TOTAL SCORE:				

Modified from: Naranjo CA et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30: 239-245.

ANNEXURE 6 - RegiSCAR criteria

It comprises of the following essential criteria :

1. Hospitalization

2. Reaction suspected to be drug related

3. Acute skin rash

In addition, the patient has to have at least 3 of the following asterisked criteria.

4. Fever more than 38°C *

5. Enlarged lymph nodes at two or more sites*

6. Involvement of at least one internal organ*

7. Haematological abnormalities * (leukocytosis, lymphocytosis, lymphocytopenia, eosinophilia or thrombocytopenia)

ANNEXURE 7 - AGEP VALIDATION SCORE BY THE EUROSCAR STUDY GROUP

Sidoroff et al.

Table 2. AGEP validation score of the EuroSCAR study group

Morphology	
Pustules	
Typical*	2
Compatible**	1
Insufficient***	0
Erythema	
Typical	2
Compatible	1
Insufficient	0
Distribution/pattern	
Typical	2
Compatible	1
Insufficient	0
Postpustular desquamation	
Yes	1
No/insufficient	0
Course	
Mucosal involvement	
Yes	≥ 2
No	0
Acute onset (≤ 10 d)	
Yes	0
No	≥ 2
Resolution ≤ 15 days	
Yes	0
No	≥ 4
Fever ≥ 38 °C	
Yes	1
No	0
PNN ≥ 7000/mm^3	
Yes	1
No	0
Histology	
Other disease	≤ 10
Not representative/no histology	0
Exocytosis of PNN	1
Subcorneal and/or intraepidermal *non* spongiform or NOS pustule(s) *with* papillary edema or subcorneal and/or intraepidermal *spongiform* or NOS pustule(s) *without* papillary edema (NOS = not otherwise specified)	2
Spongiform subcorneal and/or intraepidermal pustule(s) *with* papillary edema	3

Interpretation: ≤ 0: no AGEP, 1–4: possible, 5–7: probable, 8–12: definite.
Remarks: Patients are not included in the study, if only localized pustules are reported, the pustular rash already lasts longer than 3 weeks or a clear alternative diagnosis has been made by a dermatologist.
*Typical: typical morphology as described in the "clinical features" section
**Compatible: not typical, but not strongly suggestive of other disease.
***Insufficient: lesions can not be judged (mostly because of late stage of the disease or poor quality of pictures).

ANNEXURE 8 – PROFILE OF CADR INCLUDED IN DIFFERENT STUDIES

Pudukadan D, et al: Adverse cutaneous drug reactions

Table 1: Types of drug eruptions

Type of drug eruption	Frequency (%)
FDE	28 (31.1)
Maculopapular	11 (12.2)
SJS-TEN	17(18.8)
Urticarial	7 (7.8)
Psoriasiform	6 (6.7)
Erythema multiforme	6 (6.7)
Lichenoid	4 (4.4)
Exfoliative dermatitis	3 (3.3)
Acneiform	3 (3.3)
Acute generalized exanthematous pustulosis	2 (2.2)
Angioedema	1 (1.1)
Eczematoid	1 (1.1)
Pityriasiform	1 (1.1)
Total	**90 (100)**

Type	No. of patients	Percentage
Maculopapular rash	173	34.6
FDE	150	30
Urticaria	70	14
TEN	33	6.6
SJS	24	4.8
Erythema multiforme	22	4.4
Erythroderma	9	1.8
Lichenoid drug reaction	4	0.8
Bullous drug reaction	7	1.4
Photosensitive reaction	4	0.8
Others	4	0.8

Adapted from Sharma et al (7)

Drug reaction	No. of patients		
	Male	Female	Total
SJS-TEN	10	7	17
DRESS	2	5	7
Maculopapular drug rash	6	2	8
AGEP	1	0	1
FDE	3	1	4
Exfoliative dermatitis	2	0	2
EM	1	2	3
Urticaria	1	0	1
Total	26	17	43

Adapted from sasidharanpillai et al (94)

ANNEXURE 9 - DATA SHEET

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	slno	date	name	hspno	age	sex	occup	addr	state	phno	prescom	day	itch	itchdur	burn	burndur	conjles	conjlesdu	oralles	orallesdur	genle
2	1	########	Selvi.M	671585F	49	2	Housewif	5,Utharia	Tamil Nadu		2	12	1	12	2		2		2		
3	2	########	Joyce Will	633925D	43	2	Housewif	477 kamb	Tamil Nad	9.89E+09	2	30	1	30	2		2		2		
4	3	########	Pavithran	982472F	23	1	Student	16/13, Ch	Tamil Nad	9.94E+09	1	10	1	10	2		2		2		
5	6	########	Saraswati.	200008B	49	2	Housewif	98/1,Kana	Tamil Nadu		2	2	1	1	2		2		2		
6	7	########	Yeshi War	368534G	59	2	Housewif	Gaptey, P	Bhutan		1	10	2		2		2		2		
7	8	########	Papathi	348365F	38	2	Housewif	3/124, nay	Tamil nad	9.49E+09	3	3	1	3	1	3	1	1	1	1	
8	9	########	Sivagami	998159F	36	2	Salon wor	Madhugar	Tamil nad	9.44E+09	3	16	1	11	2		2		1	7	
9	10	########	Kanniyam	564684C	66	2	Housewif	411,43rd c	Tamil nadu		1	15	1	15	2		2		2		
10	11	########	Dinesh Ku	623189C	21	1	Student	97, Pillair	Tamil Nadu		1	1	1	1	2		2		2		
11	12	########	Chinnapat	985458F	32	2	Housewif	56, Palla s	Tamil Nad	9.96E+09	2	20	1	20	2		2		2		
12	13	########	Saramma	380514G	50	2	Housewif	Munnadat	Kerala	9.75E+09	1	4	1	4	2		2		1		
13	14	########	Geetha	998110F	38	2	Housewif	17/9,Kam	Tamil Nad	8.1E+09	2	14	1	14	2		2		2		
14	15	########	Abhinesh	106491G	20	1	Student	Cosimani	Tamil Nad	7.3E+09	3	8	1	4	1	8	1	8	1	7	
15	16	########	Neelavath	503912G	46	2	Housewif	Palamane	Andhra Pr	9.74E+09	3	5	2		1	3	1	3	1	3	
16	17	########	Manjula S	943429B	46	2	Housewif	Kannikapu	Tamil Nad	8.01E+09	2	4	2		2		2		2		
17	18	########	Sasikala S	868147F	41	2	Housewif	Jaynagar c	Andhra Pr	8.79E+09	2	2	1	1	2		2		2		
18	19	########	Parthiban	505764G	49	1		Kurusillap	Tamil Nad	9.99E+09	3	4	2		1	4	1	4	1	4	
19	20	########	Indumath	374098G	27	2	Housewif	Bajanaiko	Tamil Nad	8.94E+09	3	5	2		1	5	1	5	1	5	
20	21	########	Sudhakar	505699G	58	1		Perumalp	Tamil nadu		3	5	2		1	5	1	5	1	4	
21	22	########	Maheshw	140226M	35	2	Housewif	Melachen	Tamil Nadu		1	2	1	2	2		2		2		
22	23	########	Julie	718434	44	2	Housewif	12th cross	Tamil Nad	9.89E+09	1	7	1	7	2		2		2		
23	24	########	Malarkodi	808582A	38	2	Housewif		Tamil Nadu		1	2	1	1	1	1	2		2		
24	25	########	Valli	511072G	49	2	Housewif	Nammalw	Tamil Nad	9.44E+09	3	5	2		1	5	1	5	1	5	
25	26	########	Thamayan	853168D	61	2	Housewif	Kolaimedl	Tamil Nad	9.72E+09	1	2	1	2	2		2		2		
26	27	########	Manohara	511134G	56	1		Pulimedu	Tamil nad	7.64E+09	2	3	1	3	2		2		2		

	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AC
1	genles	genlesdur	fever	fevdur	cough	coughdur	dysp	dyspdur	jaun	jaundur	arth	arthdur	edema	edemadur	drug	indic	drugaed	drugabx	druganal	drugoth	pastrx
2	2		1	1	2		2		2		2		1		siddha me	cold and e	2	2	2	1	
3	2		1	30	1	20	2		2		2		2		Pantopraz	given with	2	2	2	1	
4	2		2		2		2		2		2		2		Phenytoir	mild head	1	2	2	2	
5	2		1	2	2		2		2		2		1	1	Rabies Ig,	Dog Bite	2	2	2	1	
6	2		2		2		2		2		2		2		Meropene	Catheter r	2	1	2	1	
7	2		1	3	2		2		2		2		2		Phenytoir	Brain met	1	2	2	2	
8	2		1	15	2		2		2		2		2		phenobar	seizure - c	1	2	2	2	
9	2		2		2		2		2		2		1	11	carbamaz	post herp	1	2	2	2	
10	2		2		2		2		2		2		2		Levetirace	TB mening	1	2	2	2	
11	2		1	20	2		2		2		2		1		Phenytoir	Cerebral v	1	2	2	2	
12	2		2		2		2		2		2		2		Meropene	Pseudome	2	1	2	2	
13	2		1	14	2		2		2		2		2		Phenytoir	CVT	1	2	2	2	
14	2		1		1	14	2		2		2		2		Valproate	Seizure di	1	2	2	2	
15	2		1	5	2		1	7	2		2		2		Cefixime/	Fever with	2	1	2	1	
16	2		1	10	2		1	2	2		2		2		Crystallin	Necrotizir	2	1	2	2	
17	2		1	1	2		2		2		2		2		Ofloxacin,	Acute gast	2	1	2	1	
18	2		1	7	1	7	2		2		2		2		Cefotaxim	Fever with	2	1	2	2	
19	2		1	3	2		2		2		2		1	3	Carbamaz	Persistent	1	2	2	2	
20	2		1	5	2		2		2		2		2		Phenytoir	CVT Super	1	2	2	2	
21	2		2		2		2		2		2		2		Chlorpron	Psychosis	2	2	2	1	
22	2		2		2		2		2		2		2		ATT	?TB Lympl	2	2	2	1	
23	2		2		2		2		2		2		2		Unknown	Fatigue, Malaise					
24	2		1	5	1	5	2		2		2		2		Azithro/ca	Pain at Hy	1	1	2	2	
25	2		2		2		1	6	2		2		2		B.Penicilli	Necrotizir	2	1	2	1	
26	2		1	8	2		1	8	2		2		2		hydralazir	cellulitis,I	2	1	2	1	

	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH
1	drugoth	pastrxn	typorxn	ster	sterdurn	indicster	comorb	diab	htn	othcomor	descomor	alcohol	alcdurn	alcqty	febrile	hr	rr	bpsys	bpdia	In	Insite
2	1	2		2			1	2	2	1	eczema	2			2	1		120	80	2	
3	1	2		1		Drug rash	1	1	2	1	Vascular h	2			2	1		120	80	2	
4	2	2		2			1	2	2	1	mild head	3			2	1	23	130	80	1	
5	1	2		1	2		2					2			2	1	22	126	88	2	
6	1	2		1	1	Drug Rash	1	2	1	1	CKD Stage	3			2	1		130	80	2	
7	2	2		1	1	drug rash	1	2	2	1	Carcinoma	2			2	1	18	100	80	2	
8	2	2		1	14	drug rash	2					2			2	1	24	100	70	2	
9	2	2		1	1	drug rash	1	2	2	1	post herp	2			2	1		110	70	2	
10	2	1	7	1	1	Drug Rash	1	2	2	1	TB Mening	3			2	1		120	80	2	
11	2	2		1	1	Drug Rash	1	2	2	1	Postpartu	2			2	1		120	80	1	
12	2	2		1	3		1	2	2	1	Pure whit	2			2	1	24	90	60	2	
13	2	2		1	1	Cerebral E	1	2	2	1	CVT,SSS th	2			2	1		110	70	2	
14	2	2		1	1	Drug Rash	1	2	2	1	Adjustme	3			2	1		100	60	2	
15	1	2		1	1	Drug Rash	1	2	2	1	febrile illr	2			1	3	22	100	60	2	
16	2	2		1	2	Drug rash	1	2	2	1	UTI, Hepat	2			1	1	18	124	86	2	
17	1	1	9	2			1	2	1	1	BPPV	2			1	1		110	60	1	
18	2	2		2			1	1	2	2		2			1	1		120	80	2	
19	2	2		2			2					2			1	2		120	80	2	
20	2	2		1	1	Drug rash	1	2	2	1	Cortical ve	1	10		1	2	24	100	70	2	
21	1	2		2			1	2	2	1	Psychosis	2			2	1		100	60	2	
22	1	2		2			1	2	2	1	Hypothyro	2			2					2	
23		1	8	1	1	Drug Rash	1	2	2	1	Bronchial	2			2					1	
24	2	2		1	1	Drug Rash	1	1	1	2		2			2	2		130	80	2	
25	1	2		1		Iatrogenic	1	1	1	1	?Obstruct	2			2	2		120	80	2	
26	1	2		2			1	2	1	1	cellulitis,	2			2	2		110	90	2	

	BG	BH	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA
1	In	Insites	rs	rsdes	gi	gides	eryth	mac	pap	macpap	purp	targ	vesbul	pust	eros	othles	descles	bsa	scaling	palm	morpl
2	2		1		1		2	2	1	2	2	2	2	2	2	1	flexural ac	3	2	2	
3	2		1		1		2	2	2	1	2	2	2	2	2	2		3	2	2	
4	1	1	1		1		2	1	2	1	2	2	2	2	2	2		2	2	2	
5	2		1		1		1	1	2	2	2	2	2	2	2	2		3	2	1	Erythe
6	2		2	Decreased	1		1	2	2	1	2	2	2	2	2	1	desquama	3	1	2	
7	2		1		1		2	2	1	2	2	1	1	2	1	2		1	2	1	Erythe
8	2		1		1		2	2	1	2	2	2	2	2	2	2		3	1	2	
9	2		1		1		1	2	2	2	2	2	2	2	2	2		2	1	1	scalin
10	2		1		1		2	2	1	2	2	2	2	2	2	2	dusky ery	2	2	2	
11	1	4	1		1		1	2	2	2	2	2	2	2	2	1	scaling	3	1	1	Desqu
12	2		1		1		1	2	2	1	2	2	2	2	2	2		1	2	2	
13	2		1		1		1	1	1	2	2	2	2	2	2	2		2	1	2	
14	2		1		1		2	2	1	2	1	1	1	2	1	2		2	2	1	Purpu
15	2		2	Left sided	1		2	2	2	2	2	1	1	2	1	2		3	2	1	Target
16	2		1		2	hepatome	1	2	2	1	2	2	2	2	2	2		3	2	2	
17	1	1	1		1		2	2	1	1	2	2	2	2	2	2		3	2	2	
18	2		1		1		2	2	1	2	2	2	2	2	1	2		1	2	2	
19	2		1		1		2	2	2	2	2	1	1	2	1	2		1	2	1	Atypic
20	2		1		1		2	2	1	2	1	1	2	2	1	2		1	2	2	
21	2		1		1		2	2	1	2	2	2	1	2	2	1	photodist	1	2	2	
22	2		1		1		2	2	2	2	2	2	2	2	2	1	Excoriatio	1	2	2	
23	1	1	1		1		2	2	2	2	2	2	1	2	2	1	Infiltrated	1	2	1	Bullou
24	2		1		1		2	2	2	2	2	1	1	2	1	2		3	2	2	
25	2		2	Decreased	1		1	1	1	2	2	2	2	2	2	2		2	2	2	
26	2		1		1		1	1	2	2	1	1	1	1	1	1	infiltrated	2	1	2	

	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP	CQ	CR	CS	CT	CU
1	morphpal	sole	morphsol	face	faceles	eyeles	oral	genital	nail	nailchan	hair	hairchan	hb	plt	tc	neutr	lymph	eosin	mono	baso	tb
2		2		1	facial ede				2		2		10.4	283000	14300	51	22	18	9	0	
3		2		2					2		2		13.8	378000	12100	74	12	11	3	0	
4		2		2					2		2		15.1	302000	7100	57	22	10	9	2	
5	Erythemat	2		1	Facial Ede				2		2		12.5		13400	82	15	0	3	0	
6		2		1	Facial ede				2		2		8.3	326000	8700	79	10	8	3	0	
7	Erythema	1	erythema	1	atypical ta	congestio	superficia		2		1	atypical ta	13.9	210000	6000	68	7	16	9	0	
8		2		1	facial ede	icterus an	cheilitis w		2		2		9.6	62000	13100	42	25	0	12	0	
9	scaling	1	scaling	1	centrofaci		cheilitis		2		2		10.3	395000	11300	55	30	6	8	1	
10		2		2						2	2		14.7	240000	6100	56	17	17	10	0	
11	Desquama	1	Desquama	1	Facial eryt				2		1	Scaling wi	11.4	254000	4700	57	31	1	11	0	
12		2		1	Erythema		oral candi		2		2		8.2	163000	600	3	95	0	2	0	
13		2		2					2		2		7.5	322000	15200	66	16	8	10	0	
14	Purpuric n	1	Purpuric n	2		Eye discha	Erosions v		2		2		14	278000	14300	73	7	5	14	1	
15	Targetoid	1	Targetoid	1	Periorbita	bilateral e	Erosions c		2		2		14.3	68000	1400	24	76	0	0	0	
16		2		1	Erythema				2		2		9	270000	8600	72	16	4	8	0	
17		2		1	Erythemat				2		2			389000	11400	73	20	4	3	0	
18		2		1	facial ede	Eye conge	Erosions c		2		2		15.8	412000	14100	76	17	1	6	0	
19	Atypical ta	1	Atypical ta	1	Facial Ede	Increased	hemorrha	Erosions i	2		2		10.8	223000	5200	59	27	0	14	0	
20		2		2		severe co	mucopuru		2		2		14.5	214000	6000	47	24	1	28	0	
21		2		2					2		2		11.6		6600	55	21	11	13	0	
22		2		2					2		2		12.2	367000	8100	64	24	5	7	0	
23	Bullous le	2		1	Facial Ede				2		2		12.7	331000	8100	56	30	8	6	0	
24		2		1	Erosions a	Congestio	Collapsed		2		2		14.2	171000	5000	77	15	0	8	0	
25		2		1	Blanching				2		2		8.4	330000	9700	90	7	1	2	0	
26		2		2					2		2		10.5	389000	22300	84	10	3	3	0	

	CU	CV	CW	CX	CY	CZ	DA	DB	DC	DD	DE	DF	DG	DH	DI	DJ	DK	DL	DM	DN	DC
1	tb	prt	alb	sgot	sgpt	alphos	creat	urineosin	cxr	cxrdes	hiv	grnlsn	rptgrn	rptgrnlsn	bx	hyperk	orthok	parak	spong	spongves	lymex
2	0.7	6.8	3.8	15	25	53	0.64		1		3	0.1	2		1	1	2	1	1	2	
3	0.6	6.6	4.1	22	51	82	0.73	2	1		2	0.1	2		1	2	1	2	1	2	
4	0.4	7	4.4	21	26	101	0.9	2	1		3	0.14	2		1	2	1	2	2	2	
5	0.5	6.6	3.5	21	14	71		2			2	0.1	2		1	2	1	2	1	2	
6	0.3	5.8	2.8	16	10	149	5.96	2			2	0.16	2		1	1	2	1	1	2	
7	0.3	6	3.2	44	28	114	0.53		2	ill definec	2	0.15	2		1	1	2	2	1	2	
8	12.8	4.3	1.7	283	228	382	0.74				2	0.9	2		1	1	2	1	1	2	
9	0.3	8.1	3.8	20	12	129	0.58	1	1		3	0.67	2		1	2	1	1	2	2	
10	0.7	7.1	4.3	25	20	50	0.93	2	1		2	0.1	2		1	2	1	2	1	1	
11	0.2	6.8	4.1	18	16	161	0.52	2	1		2	0.67	2		2						
12	0.8	7.5	2.3	38	32	63	0.65		2	chronic PE	2	0.1	2		1	2	1	2	2	2	
13	0.5	6	2.6	105	59	460	0.29				2	0.15	2		2						
14	0.5	8.1	4.1	35	22	70	0.67		1		3	0.15	2		1	2	1	1	1	2	
15	0.7	4.8	1.9	483	337	400	0.73		2	b/l UZ, R L	2	4	1	4	1	2	1	2	2	2	
16	0.5	5.7	2.7	28	24	80	0.43	1	2	bilateral s	2	0.24	2		1	2	1	2	1	2	
17	0.4	6.7	4.3	14	9	81	0.72	2	1		2	0.11	2		1	2	1	2	2	2	
18	0.8	7	4.2	15	26	94	0.92		1		2	0.1	2		1	1	2	2	2	2	
19	0.7	6.9	4	20	21	132	0.62		1		2	0.12	2		2						
20	0.9	6.9	3.9	47	78	390	0.84	2	1		2	0.15	2		2						
21	0.2	7.6	4	39	70	86		2			3	0.14	2		2						
22	0.3	7	4.1	20	17	81		2			2	0.17	2		2						
23	0.2	6.5	4.1	17	16	66	0.7	2	1		2	0.1	2		2						
24	0.5	7.2	3.6	48	69	59	0.89		1		2	0.29	2		2						
25				27	8		0.7		1		2	4	2		1	2	1	2	2	2	
26	2.2	7.5	3.9	18	27	120	1.02				2	4	2		2						

	DO	DP	DQ	DR	DS	DT	DU	DV	DW	DX	DY	DZ	EA	EB	EC	ED	EE
1	lymexo	basvac	dermede	necker	neutinf	lymphinf	eosinf	histinf	plasminf	mastinf	othinf	descinf	cleft	othhpe	fnldx		
2	1	2	2	1	2	1	1	1	2	1	2		2		Maculopapular Exanthem		
3	1	1	2	1	1	1	1	1	2	1	2		2		Maculopapular Exanthem		
4	2	1	2	1	1	1	1	2	1	2	2		2	Occasiona	Maculopapular Exanthem		
5	2	2	1	2	2	1	1	1	2	2	2		2		Maculopapular Exanthem		
6	1	2	1	2	1	1	1	1	2	2	2		2		Maculopapular Exanthem		
7	1	2	1	1	1	1	1	2	2	2	2		1	epiderma	SJS		
8	1	1	2	1	2	1	1	1	2	1	2		2		DRESS		
9	1	2	1	2	1	1	1	1	2	2	2		2		Maculopapular Exanthem		
10	2	2	1	1	1	1	1	1	2	2	2		2		Maculopapular Exanthem		
11															Drug Induced Erythroderma		
12	2	2	1	2	2	1	1	1	2	1	2		2		Maculopapular Exanthem		
13															DRESS		
14	1	1	1	1	2	1	1	1	2	2	2		2	Mild muci	Erythema Multiforme Major		
15	1	1	1	1	1	1	1	1	2	2	2		1	No viral in	TEN		
16	1	1	1	2	2	1	1	1	2	1	2		2		Maculopapular Exanthem		
17	2	2	2	2	2	1	2	1	2	2	2		2		Maculopapular Exanthem		
18	2	2	2	2	2	1	2	1	2	2	2		2	Increased	Erythema Multiforme Major		
19															SJS		
20															SJS		
21															Maculopapular Exanthem		
22															Maculopapular Exanthem		
23															Bullous FDE		
24															TEN		
25	2	2	2	2	1	1	2	1	2	2	2		2		Maculopapular Exanthem		
26															Maculopapular Exanthem		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
25	26	########	Thamayar	853168D	61	2	Housewif	Kolaimedl	Tamil Nad	9.72E+09	1	2	1	2	2		2		2		
26	27	########	Manohara	511134G	56	1		Pulimedu	Tamil nad	7.64E+09	2	3	1	3	2		2		2		
27	28	########	Venkatesl	763499F	47	1		Marichett	Andhra Pr	9.57E+09	2	7	1	6	2		2		2		
28	29	########	Protiva Ra	512762G	53	2	Housewif	Nazarpur,	Bangladesh		3	3	2		1	7	1	7	1	7	
29	30	########	Anita	511840G	39	2	Housewif	Rompiche	Andhra Pr	9.67E+09	3	8	1	2	2		2		1	2	
30	31	########	Sundarase	620464D	67	1		Kevin Vill	Tamil nad	9.44E+09	2	4	1	4	2		2		2		
31	32	########	Munuswa	523264G	58	1		Pillairkoil	Tamil nad	9.84E+09	3	6	1	5	2		1	4	1	5	
32	33	########	Jyotsna M	651570G	79	2	Housewif	Tamluk, P	West Beng	8.3E+09	1	1	1	1	2		2		2		
33	34	########	Shyamapa	773368D	65	1	Retired	Gopinath	West Beng	9.44E+09	2	2	1	2	2		2		2		
34	35	########	Mythili	522045G	22	2	Housewif	Ramarkoil	Tamil nad	7.6E+09	3	6	2		2		2		1	5	
35	36	########	Jayakodi	927895B	47	2	Housewif	Vedhuruk	Andhra Pr	9.96E+09	2	2	1	2	2		2		2		
36	37	########	Sunkesula	527433G	30	1	auto drive	Ahmad na	Andhra Pr	9.1E+09	2	4	2		2		2		2		
37	38	########	Padmavat	657404C	51	2	Housewif	51st stree	Tamil nad	9.79E+09	2	6	1	6	2		2		2		
38	39	########	Anandha	531013G	50	2	Housewif	Narimuru	Tamil nad	9.87E+09	3	4	2		2		2		1	4	
39	40	########	Nasima Al	531144G	44	2	Housewif	Thonthon	Bangladesh		3	10	2		1	10	2		1	10	
40	41	########	Suresh	532581G	41	1	Autodrive	Santhinag	Tamil nad	9.63E+09	1	3	1	3	2		2		2		
41	42	########	Muniswar	744833G	58	1	Manual la	Mariamm	Tamil nad	9.35E+09	1	6	1	6	2		2		2		
42	43	########	Gandham	536845G	37	2	Housewif	Rajampet	Andhra Pradesh		2	9	1	4	2		2		2		
43	44	########	Nirosha P	394763G	28	2	Housewif	Janappa S	Tamil nad	8.88E+09	3	5	2		2		2		1	5	
44	45	########	Jaganatha	245848B	37	1	Farmer	venkanna	Tamil nadu		2	11	1	10	1	15	2		1		
45	46	########	Iswarya	777857G	20	2	Flower ve	Vaniyan S	Tamil nad	9.79E+09	3	1	1	1	1	1	2		1	1	
46	47	########	Vijayalaks	727349D	30	2	Housewif	North st,P	Tamil nad	9.37E+09	3	2	2		2		1	2	1	1	
47	48	########	Bharathi	858246G	20	2	Housewif	Bajanaiko	Tamil nad	8.53E+09	3	6	1	6	2		2		1	3	
48	49	########	Alpana Ch	557881G	65	2	Housewif	Chatterje	West Beng	9.23E+09	3	3	2		2		1	3	1	3	
49	50	########	Eswari Pa	796838B	70	2	Housewif	Sriramnag	Orissa	9.24E+09	2	3	2		1	2	2		2		

	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AC
25	2		2		2		1	6	2		2		2		B.Penicilli	Necrotizir	2	1	2	1	
26	2		1	8	2		1	8	2		2		2		hydralazir	cellulitis,L	2	1	2	1	
27	2		1	6	2		2		2		2		2		dapsone	pyoderma	2	2	2	1	
28	2		1	45	1	15	1		2		2		2		Amoxicla	Fever with	2	1	2	2	
29	2		1	2	2		2		2		1	2	1	2	carbamaz	neuromye	1	2	2	1	
30	2		1	2	2		2		2		2		2		meropen	NFGNB se	2	1	2	2	
31	2		1	3	1	2	2		2		2		2		phenytoir	CVA with	1	2	2	2	
32	2		2		2		2		2		2		2		cloxacillin	infected i	2	1	2	2	
33	2		1	2	2		2		2		2		1	60	cefazolin/	staphyloc	2	1	2	1	
34	2		1	6	1	15	1	15	2		2		1	4	magnex	?pneumo	2	1	2	2	
35	2		1	3	2		2		2		2		1		magnex/p	APML, NF	2	1	2	1	
36	2		1	15	2		2		1	20	2		2		meropen	high grade	2	1	2	2	
37	2		1	90	2		2		2		2		2		carbamaz	NMO, UTI	1	1	2	2	
38	2		1	1	2		2		2		2		2		Carbamaz	post herp	1	2	2	2	
39	2		1	1	2		2		2		2		2		phenyt/pl	?meningi	1	2	2	1	
40	2		2		2		2		2		2		2		phenytoir	seizures	1	2	2	2	
41	2		2		2		2		2		2		2		augmentii	LRI	2	1	2	1	
42	2		1	9	2		2		2		2		1	4	sulfasalaz	inflammat	2	2	2	1	
43	2		2		2		2		1	15	2		2		pantop/o	UGI bleed	2	1	2	1	
44	2		1	2	2		2		2		2		2		carbamaz	seizure di	1	2	2	2	
45	2		1		2		2		2		2		1		carbamaz	mania,psy	1	2	2	2	
46	2		2		2		2		2		2		2		phenytoir	GTCS	1	2	2	2	
47	2		1	5	2		2		2		2		2		carbamaz	seizure di	1	2	2	2	
48	2		1	90	2		2		2		2		2		meropen	pyelonepl	2	1	2	1	
49	2		1	2	2		2		2		2		1	2	Penidure,	prophylax	2	1	2	2	

	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI
25	2		1		Iatrogenic	1	1	1	1	?Obstruct	2			2	2		120	80	2		
26	2		2			1	2	1	1	cellulitis,	2			2	2		110	90	2		
27	2		2			1	2	2	1	pyoderma	3			1	2	20	120	72	1	3	
28	2		2			1	2	2	1	GNB Sepsi	2			1	2	24	100	60	2		
29	2		1	1	drug rash	1	2	2	1	NMO spec	2			1	1	22	110	70	2		
30	2		2			1	1	1	1	CKD,HBV+	3			1	3		90	60	2		
31	2		1	1	drug rash	1	2	1	1	CVA with	1	15		1	1	28	100	60	2		
32	2		2			1	2	2	1	AKI, left ir	2			2					2		
33	1	8	2			1	2	1	1	COPD, LVH	2			2	1		140	80	1	2	
34	2		1	2	acute ons	1	2	2	1	acute pro	2			1	1	26	130	70	2		
35	2		2			1	2	2	1	RA, acute	2			2	1	20	110	70	1	2	
36	2		2			1	2	2	1	acute nec	1	3	3500	2	3		110	80	2		
37	2		1	3	NMO spec	1	2	2	1	NMO, ?IBI	2			1	1		140	90	2		
38	2		1	1	drug rash	1	1	1	2		2			2	1		120	70	2		
39	2		1	45	space occ	1	1	2	1	PE,intracra	2			2	1	26	100	60	2		
40	2		2			1	2	2	1	seizures s	1	2	5250	2	1		120	70	2		
41	2		2			1	1	2	1	bronchial	3			2	1	18	140	80	2		
42	2		1	1	drug rash	1	1	2	1	inflammat	2			2	1		120	80	2		
43	2		1	2	drug rash	1	2	2	1	acute fatty	2			2	1		110	70	2		
44	2		2			1	2	2	1	seizure di	3			1	1	20	110	80	2		
45	2		2			1	2	2	1	mania, psy	2			2					2		
46	2		2			1	2	2	1	SLE,class I	2			2	2		100	70	2		
47	2		2			1	2	2	1	seizure di	2			2	1	14			1	2	
48	2		2			1	1	1	1	hypothyro	2			1	1	30	110	74	2		
49	1	9	2			1	2	1	1	AKI,chron	2			1	2	20	100	80	2		

	BI	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC
25	2	Decreased	1		1	1	1	2	2	2	2	2	2	2		2	2	2		2	
26	1		1		1	1	2	2	1	1	1	1	1	1	infiltrated	2	1	2		2	
27	1		1		2	2	2	1	2	2	2	2	2	1	infil papul	3	2	2		2	
28	2	Decreased	1		2	2	2	2	1	1	1	2	1	2		3	2	2		2	
29	1		1		1	2	2	1	2	2	2	2	2	1	crusting	1	2	2		2	
30	2	occasiona	1		2	2	1	2	2	2	2	2	2	2		3	2	2		2	
31	1		1		2	2	2	2	2	1	1	2	1	2		2	2	2		2	
32	1		1		2	2	1	2	2	2	2	2	2	2		1	2	2		2	
33	1		1		1	2	2	2	2	2	2	2	2	1	Scaling	3	1	1	diffuse co	1	diffus
34	2	crepts in F	1		1	2	2	1	2	2	2	2	2	2		2	2	1	blanching	1	blanch
35	1		1		1	2	2	2	1	2	2	2	2	1	urticated	3	2	2		2	
36	1		2	distended	1	1	2	2	2	2	2	2	2	2		2	2	2		2	
37	1		1		1	2	1	1	2	2	2	2	2	1	flexural u	3	2	2		2	
38	1		1		2	2	2	2	2	1	2	2	1	2		1	2	2		2	
39	1		1		2	2	2	2	1	1	1	2	1	2		3	2	1	flaccid bul	1	flaccic
40	1		1		2	2	2	1	2	2	2	2	2	1	infiltrated	3	2	2		2	
41	2	wheeze ir	1		2	2	2	1	2	2	2	2	2	1	infiltrated	3	2	2		2	
42	1		1		2	2	2	1	2	2	2	2	2		infiltrated	3	1	2		2	
43	1		1		2	2	2	2	2	1	1	2	1	2		2	2	2		1	petec
44	1		1		2	2	1	2	2	1	2	2	2	2		3	2	2		1	papul
45	1		1		2	2	1	2	2	1	2	2	2	2		3	2	1	erythema	2	
46	1		1		2	2	1	2	1	1	2	2	1	1	crusted er	1	2	1	purpuric r	2	
47	1		1		1	2	2	2	2	2	2	2	2	2		2	1	2		2	
48	1		1		2	2	2	2	1	1	1	2	1	1	reticulate	3	2	2		2	
49	1		1		1	2	2	2	2	2	1	1	1	1	sterile pir	3	2	2		2	

	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP	CQ	CR	CS	CT	CU	CV	CW
25		1	Blanching				2		2		8.4	330000	9700	90	7	1	2	0			
26		2					2		2		10.5	389000	22300	84	10	3	3	0	2.2	7.5	
27		2				phimosis	2		2		13.6		8300	68	15	12	5	0			
28		1	atypical ta	mild cong	lips cruste		2		2		10.9	45000	4700	34	61	0	4	1	0.7	4.9	
29		1	facial ede		erosions c		2		2		11.9	211000	5900	64	20	3	13	0	0.2	6	
30		2					2		2		9.2	149000	4100	72	12	10	6	0	0.4	6.9	
31		2		bilateral e	crusting o	herpetic u	2		2		12.3	190000	5600	63	10	19	8	0	0.5	7.2	
32		2					2		2			186000	15800	82	8	0	10	0			
33	diffuse co	2					1	b/l thumb	1	diffuse sc	10.4	320000	13400	57	10	23	8	0	0.2	6.6	
34	blanching	1	desquama		few discre		2		2		8.2	51000	900	40	40	0	20	0	1.1	7.3	
35		1	facial ede				2		2		8.9	49000	5900	90	6	1	0	0	0.6	7.5	
36		2					2		2		8.4	395000	39900	89	4	2	3	0	13.7	5.3	
37		1	perioral,p				2		2		8.7	246000	1700	72	12	0	16	0	0.5	4.3	
38		2			h'gic crust	discrete e	2		2		12	26000	6700	71	22	1	6	0	0.5	7.6	
39	flaccid bul	2			flaccid bul		1	acute parc	2		8	325000	6400	86	9	1	4	0	0.4	7.3	
40		2					2		2		18.8	348000	6600	53	21	13	12	1	0.3	8.3	
41		2					2		2		16	293000	17300	71	10	13	6	0			
42		1	facial ede				2		1	diffuse sc	12.4	69000	14300	62	22	6	10	0	0.7	7.7	
43	petechiae	2			h'gic crust		2		2		9.3	15000							13.5	4.6	
44	papular le	2			cheilitis		2		2		17.2		4900	43	27	15	14	1	0.5	7.3	
45		1	facial ede		crusted pl		2		2		11.6	250000	9100	63	16	9	12	0			
46		2		moderate	crusted er		2		2		13	236000	9900	83	9	2	6	0	0.2	7.2	
47		1	desquama		cheilitis		2		2		13.5	216000	4800	60	23	7	10	0	0.2	6.6	
48		1	purpuric n	congestio	h'gic crust		2		2		7.8	74000	5900	84	6	2	8	0	1.1	4.6	
49		1	facial ede		cheilitis		2		2		15.3	312000	21600	96	2	0	2	0	0.7	7	

	CW	CX	CY	CZ	DA	DB	DC	DD	DE	DF	DG	DH	DI	DJ	DK	DL	DM	DN	DO	DP	DQ
25		27	8		0.7		1		2	4	2		1	2	1	2	2	2	2	2	
26	3.9	18	27	120	1.02				2	4	2		2								
27			15		0.93	2	1		2	0.24	2		2								
28	2	161	85	59	0.74		2	Left pleur	2	0.5	2		2								
29	3.5	30	41	69	0.9	2	1		2	0.33	2		2								
30	2.9	27	5	73	9.01				2	1	2		2								
31	3.5	64	33	84	2.01		2	bilateral i	2	0.24	2		2								
32		182	106		1.31	2	1		2	0.21	2		2								
33	3.8	26	12	103	1.02	2			2	0.1	2		1	1	1	1	1	2	1	1	
34	4.3	21	27	91	0.74	1	2	bilateral l	2	0.14	2		2								
35	4.4	29	31	123	0.59		1		3	0.13	2		2								
36	1.8	70	52	80	1.76		1		3	0.18	2		2								
37	2.2	24	27	96	0.52		1		2	0.3	2		1	2	1	2	1	2	1	2	
38	4	19	27	46	0.57		2	R>L pleura	2	0.08	2										
39	3.3	42	34	42	3.01		2	suboptima	2	0.3	2		2								
40	4.6	31	24	113	0.72	2	1		2	0.29	2		2								
41		13	19		0.79		1		3	0.2	2		2								
42	4.6	88	144	131	0.69	2	1		2	0.5	2		1	1	2	1	1	2	1	2	
43	2	72	43	175	0.67		1		2	0.11	2		1	2	1	1	2	2	1	2	
44	4.7	159	256	178	0.8	2	1		2	0.21	2		1	1	2	2	1	2	1	1	
45		25	31		0.57	2			3	0.19	2		2								
46	3.8	95	155	122	0.86		2	well defir	2	0.26	2		1	1	2	2	1	2	1	1	
47	4	61	20	57	0.65	2			3	0.56	2		2								
48	1.7	25	4	99	2.79		1		2	0.46	2		1	1	2	1	2	2	1	1	
49	3.4	22	10	278	0.71		2	cardiomeg	2	0.1	2		1	2	1	1	1	2	2	2	

	DP	DQ	DR	DS	DT	DU	DV	DW	DX	DY	DZ	EA	EB	EC	ED	EE
25	2	2	2	1	1	2	1	2	2	2		2		Maculopapular Exanthem		
26														Maculopapular Exanthem		
27														DRESS		
28														TEN		
29														SJS		
30														Maculopapular Exanthem		
31														SJS-TEN overlap		
32														Maculopapular Exanthem		
33	1	1	1	1	1	1	1	1	2	2		2		Drug Induced Erythroderma		
34														Maculopapular Exanthem		
35														Maculopapular Exanthem		
36														Maculopapular Exanthem		
37	2	1	1	2	1	1	1	2	2	2		2		Maculopapular Exanthem		
38														Erythema Multiforme Major		
39														TEN		
40														Maculopapular Exanthem		
41														Maculopapular Exanthem		
42	2	1	1	2	1	1	1	1	2	2		2	occ ill defi	DRESS		
43	2	1	1	1	1	1	1	1	2	2		1		SJS-TEN overlap		
44	1	2	1	1	1	2	1	2	2	2		2		DRESS		
45														Erythema Multiforme Major		
46	1	2	1	2	1	1	1	2	2	2		2		SJS		
47														DRESS		
48	1	1	1	1	1	1	1	2	2	2		1	no definit	TEN		
49	2	2	1	1	1	1	1	2	2	2		2	subcornea	AGEP		